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DECEMBER, 1956

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SPECIAL SMUT RESEARCH NUMBER

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FURTHER STUDIES ON THE SYNONYMY AND HOST RANGE OF THE DWARF BUNT FUNGUS, *TILLETIA CONTRAVERSA**

RUBEN DURAN AND GEORGE W. FISCHER†

In 1952 Fischer (4) described as new the smut fungus causing dwarf bunt of wheat and *Agropyron* spp., giving it the binomial, *Tilletia brevifaciens.* Conners (2), in 1954, comparing the type of *T. brevifaciens* with the type and other collections of *T. contraversa* Kühn on *Agropyron* spp. in Europe, concluded that the two are synonymous and that the dwarf bunt fungus should be referred to the earlier binomial, *T. contraversa.* This conclusion has stimulated us to make a similar comparison of the dwarf bunt fungus with other species of *Tilletia* on cereals and grasses. These observations are part of a critical study of the comparative morphology of many hundreds of collections of the genus *Tilletia* from all over the world. A number of collections have thus come to light which add to our knowledge of the synonymy and host range of the morphologic species, *T. contraversa.* A brief report on most of these collections has appeared elsewhere (3); the present paper is a more detailed account, plus some additional observations on collections subsequently studied.

The new hosts and new synonymy that have been revealed in the present studies are summarized in Table I. If our interpretations are correct, at least two other species and probably a third are add-

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† Research Assistant and Plant Pathologist, respectively, Washington Agricultural Experiment Stations.

TABLE I. NEW HOSTS AND SYNONYMY FOR THE DWARF BUNT ORGANISM, TILLETIA CONTRAVERSA

Host	Locale	Original Determination
Aegilops umbellata	Turkey	Tilletia tritici
Aegilops cylindrica	Iraq	Tilletia tritici
Aegilops triuncialis	Iraq	Tilletia tritici
Alopecurus agrestis	Italy	Tilletia calospora (Type)
Bromus erectus	Bohemia	Tilletia guyotiana
Elymus crinitus	Yugoslavia	Tilletia bornmülleri
Hordeum bulbosum	U.S.S.R. Syria Turkestan Turkey Yugoslavia	Tilletia hordei, Tilletia pancicii, Tilletia brevifaciens
Hordeum leporinum	Iran Australia Algeria Turkey Spain	Tilletia caries, Tilletia hordei
Hordeum vulgare	Bulgaria Japan Yugoslavia Iran Iraq	Tilletia hordei, Tilletia pancicii (Type)
Lolium remotum	Denmark	Tilletia lolii

ed to the synonymy of the dwarf bunt fungus. Ciferri (1) considered one of these, *Tilletia calospora* Pass., synonymous with *T. contraversa* 18 years ago. The close relationship of *T. pancicii* Bub. and Ran. on *Hordeum* spp., to *T. contraversa* appears to have gone unnoticed. A comparison of the types of *T. pancicii* with the type of *T. contraversa,* however, (Fig. 1) can leave little doubt of this relationship. Further evidence of the relationship of *T. pancicii* to *T. contraversa* is seen in the reports of Kuribayashi and Ichikawa (8, 9) and Tasugi and Yamade (11) on the stinking smut of barley in Japan. The former authors speak repeatedly of the extreme dwarfing of the host, the problem of soil infestation, the absence of the smut in spring-sown barley, the large prominent areolae on the spores, and other physiological and morphological features that characterize *T. contraversa* on wheat and grasses. Kuribayashi and Ichikawa *(op. cit.)* assigned the stinking smut of barley in Japan to *T. pancicii.* After comparing the Japanese barley smut with the type of *T. pancicii* and the type of *T. contraversa,* we are certain that all three represent a single species.

Many collections of what had been identified as *Tilletia hordei* Körn. on *Hordeum* spp. have also been included in this study of the dwarf bunt complex. Unfortunately, we have not been able to obtain type material of this species. However, the collections we have had the opportunity to study from Europe, Asia, North Africa and Australia certainly seem to be related to *T. contraversa.* If these collections are representative of *T. hordei,* then this species should be added to the synonymy of *T. contraversa;* only an examination of the type of *T. hordei* can confirm this. Still another possible synonym to add to the *T. contraversa* complex is *Uredo (Tilletia) secalis* Corda. However, we do not know precisely what smut this binomial refers to (5). Certainly some collections labeled *Tilletia secalis* Kühn belong in *T. contraversa* (Fig. 1).

A collection of *"Tilletia bornmülleri"* on *Elymus crinitus* in Yugoslavia shows unquestionable similarity to *T. contraversa* (Fig. 2). However, a study of the type of *T. bornmülleri* showed conclusively that these two species are quite dissimilar. The collection on *E. crinitus,* then, is not *T. bornmülleri* but rather is *T. contraversa,* and adds a previously unrecognized host to the list for *T. contraversa.* The same conclusion applies to a collection of *"T. guyotiana"* on *Bromus erectus* from Bohemia. The collection is clearly not *T. guyotiana,* as a study of the type of this species quickly revealed. As can be seen from Figure 2, it is *T. contraversa.* Another collection, *"Tilletia lolii",* on *Lolium remotum* in Denmark (Fig. 2) obviously is closely related to *T. contraversa* and suggested the possibility of a synonymous relationship between these two species of *Tilletia.* Here again, however, a study of the type material of *T. lolii* showed unmistakably that the latter is a species quite distinct from *T. contraversa.* So the collection of *Lolium remotum* adds a previously unrecognized host for *T. contraversa.* The collections of *"Tilletia tritici"* on *Aegilops* spp. from Turkey and Iraq have spores with wide, deep areolae and encased in a sheath as in *T. contraversa,* and are here considered as belonging to this species. Here again, a new host genus and three more previously unrecognized host species are thus added to the list of hosts of the dwarf bunt fungus.

Collectively, the observations described above add four new genera and 10 new species to the list of recognized hosts for the dwarf bunt fungus, *Tilletia contraversa,* and two additional species of *Tilletia* to its synonymy with the strong possibility of a third *(T.*

hordei). The question may arise in the minds of some readers: Is it a reasonable conclusion, merely because these collections (Table I, Figs. 1 and 2) *look* like *Tilletia contraversa,* that they necessarily *are* of that species? To anyone who would advocate a morphological species concept and who is not impressed with any taxonomic significance of the inevitable minor morphological variations, the answer must be affirmative. This philosophy of speciation in the smut fungi has been proposed by Fischer and Shaw (6). More recently, Holton and Kendrick (7) have called attention to certain problems inherent in the recognition of dwarf bunt of wheat as caused by a species separate from that causing common bunt, *T. caries.* Their objections are chiefly based on the existence of intergrades which probably arise by natural hybridization between the two species. The latter authors do recognize the dwarf bunt fungus as a separate entity of some kind in that they acknowledge the existence of a distinct disease of wheat known as dwarf bunt, short smut, low smut, *et al.*

The recognition of the dwarf bunt fungus as a species is complicated, as is so often the case, by the relative nature of any philosophic or even practical approach to the delimitation of a species. The establishment of criteria for species delimitation is, of necessity, weakened by the artificial aspects of any system devised for the purpose. That this may be true, is no reason to cease striving for taxonomic concepts of genuine utility and practicability.

Dwarf bunt has been variously regarded as a discrete organism from that causing common bunt, as a mutant from common bunt, and as a possible hybrid between two races of common bunt. Conners (2) advanced the hypothesis that dwarf bunt of wheat arose long ago from a similar smut *(Tilletia contraversa)* on grasses related to wheat in the mountainous areas of Europe or the Caucasus range. The typical dwarf bunt of wheat and grasses of today is strikingly similar to the long-established *T. contraversa.* Thus it seems logical to use that binomial to embrace not only the wheat dwarf bunt, but also similar smuts on many wild grasses and grains in many parts of the world. Hybrids and intergrades among species are commonly encountered in many groups of plants, and are a source of frustration to discriminating taxonomists. Likewise, few species of any group of organisms are so well-defined that no perplexing variants can be found. For these reasons we still are inclined to consider Kühn's *T. contraversa* as a good but complex species, and

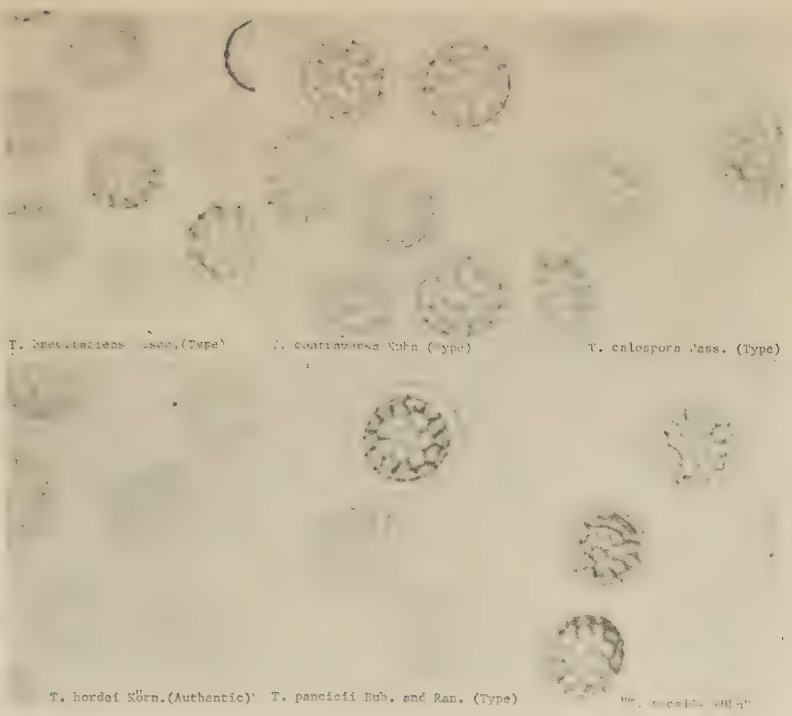
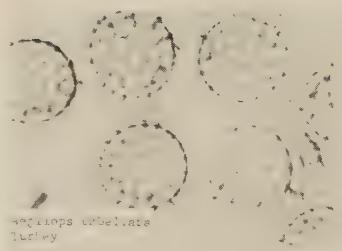
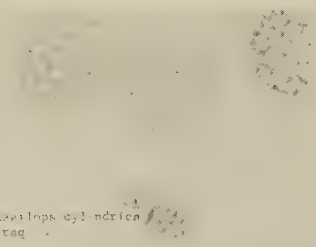


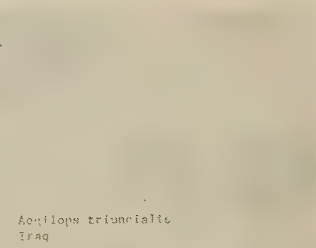
Figure 1. Photomicrographs of spores from type of authentic specimens of the species now believed to be components of the dwarf bunt (*Tilletia contraversa*) complex. The "*T. secalis* Kühn" is not type, or "authentic", but is included to illustrate one of the rye bunts believed to belong in *T. contraversa* and which is often labeled as "*T. secalis* Kühn". The relationship of the species of *Tilletia* on rye is discussed in a separate paper (5). × approx. 600. Somewhat retouched to compensate for lack of perspective and sharp focus.



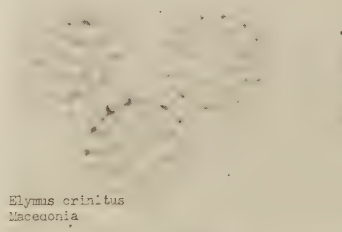
Aegilops umbellata
Turkey



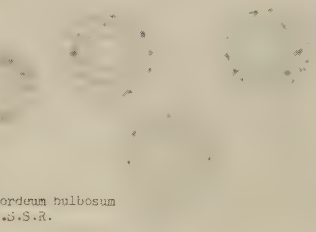
Aegilops cylindrica
Iraq



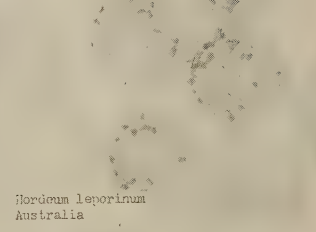
Aegilops triuncialis
Iraq



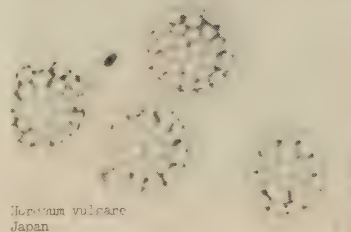
Elymus crinitus
Macedonia



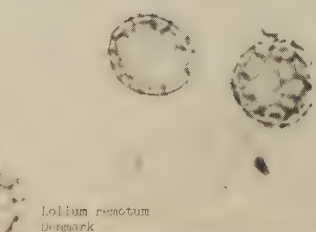
Hordeum bulbosum
U.S.S.R.



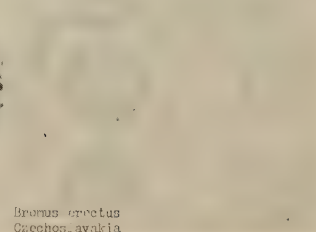
Hordeum leporinum
Australia



Hordeum vulgare
Japan



Lolium remotum
Denmark



Bromus erectus
Czechoslovakia

to include under this binomial all of the bunt fungi of similar morphology.

Much has been said about the taxonomic significance of the gelatinoid sheath which more or less encases the individual spores of the dwarf bunt fungus. It should be emphasized that the character is not unique to the dwarf bunt fungus. Recent critical study of the entire genus *Tilletia* by the authors has shown that a well-defined gelatinoid sheath around the teliospore is characteristic of many of the species. The sheath is thus a taxonomic character, but seldom of much significance *alone*. More often it serves as a supporting character with other diagnostic features such as the depth, size, and configuration of the areolae; the size, shape, and color of the mature spores; the size, shape, color and wall thickness of the sterile cells; the color of the spore mass, and other sorus characters; and the effect on the host, place of sporulation, etc. The recognition of the collective species, *Tilletia contraversa,* involves all these characters. Since the original description of *T. contraversa* did not include all the characters of the species as we recognize it today, an emended description follows, with an up-to-date list of synonyms, hosts and geographic distribution.

Tilletia contraversa Kühn—In Rabenh., Fungi Europaei. Hedwigia 13: 188-189. 1874. *emend.*

Tilletia caries Auct. p.p.
Tilletia secalis Auct. p.p.
Tilletia tritici Auct. p.p.
Tilletia calospopra Pass. Nouvo Giorn. Bot. Ital. 9: 238. 1877.
Tilletia hordei Körn. Hedwigia 16: 30. 1877.
Tilletia pancicii Bub. and Ran. Ziets. f. landw. Versuch. Oesterr. 1899: 545. 1899.
Tilletia tritici nanifca Wagner. Zietschr. Pflbau. und Pflschutz 1: 1-13. 1950.
Tilletia brevifaciens G. W. Fisch. Res. Stud. State Coll. Wash. 20: 11. 1952.

Figure 2. Photomicrographs of spores of collections of *Tilletia* that are considered to belong to the composite species, *T. contraversa,* and which add hosts not previously recognized for this smut fungus. From herbarium specimens originally determined as *T. tritici, T. hordei, T. pancicii, T. lolii, T. guyotiana, T. bornmulleri.* Retouched somewhat to provide perspective and to correct lack of sharp focus. × approx. 950.

Sori in the ovaries, usually infecting all of them, mostly globose to broadly ellipsoid but varying with different hosts, covered by the pericarp; spore mass dark grayish-brown to almost black, often rather agglutinated; host plants usually considerably stunted but varying somewhat with different hosts and perhaps with biotypes of the organism.

Sterile cells fewer and on the average smaller than the spores, regularly globose, with smooth walls, hyaline or faintly greenish or brownish, sometimes encased in a hyaline gelatinoid sheath 2-4μ thick, 9.5-21μ in diameter, including the sheath.

Spores mostly globose or subglobose, varying shades of yellowish-brown, reddish-brown or grayish-brown; exospore usually adorned with rather large regular polygonal reticulations 3-5μ in diameter, but with occasional biotypes showing irregular to subcerebriform markings, 1.5-2.5μ high and embedded in a hyaline gelatinoid sheath 1.5-4μ thick; spores in most collections 19-24μ in diameter, but occasionally as low as 17μ or as high as 26μ, including the sheath.

On Gramineae:

Aegilops cylindrica Host: Iraq
Aegilops triuncialis L: Iraq
Aegilops umbellata Zhuk.: Turkey
Agropyron cristatum (L.) Gaertn.: Caucasus, Siberia, U.S. (Idaho, Oregon)
Agropyron desertorum (Fisch.) Schult.: U.S. (Idaho, Oregon)
Agropyron diazii Sennen: Spain
Agropyron elongatum (Host) Beauv.: U. S. (Oregon)
Agropyron inerme (Scribn. & Smith) Rydb.: U.S. (Oregon)
Agropyron intermedium (Host) Beauv.: Hungary, Poland, Switzerland, U. S. (Idaho, Oregon, and Washington), Yugoslavia
Agropyron litorale (Host) Dum.: Germany
Agropyron orientale (L.) Roem & Schult.: Caucasus
Agropyron repens (L.) Beauv.: Czechoslovakia, Denmark, Germany, Poland, Siberia, Turkey, U.S.S.R., Yugoslavia
Agropyron rigidum var. *tomentosum* Regd.: Caucasus, Turkey
Agropyron sibiricum (Willd.) Beauv.: U. S. (Idaho)
Agropyron subsecundum (Link) Hitchc.: U.S. (Idaho, Oregon)
Agropyron trachycaulum (Link) Malte: U.S. (Idaho, Oregon)
Agropyron trichophorum (Link) Richt.: Persia, U.S. (Oregon)
Agropyron triticeum Gaertn.: Siberia
Alopecurus myosuroides Huds.: Italy
Arrhenatherum elatius (L.) Presl.: U.S. (Idaho, Oregon)
Bromus erectus Huds.: Czechoslavakia
Bromus marginatus Nees: U. S. (Idaho, Oregon)
Dactylis glomerata L.: U. S. (Oregon)
Elymus aralensis Regel: Turkestan
Elymus canadensis L.: U. S. (Idaho, Oregon)
Elymus crinitus Schreb.: Greece (Macedonia)
Elymus sibericus L.: U.S. (Oregon), Yugoslavia
Festuca elatior L.: U. S. (Oregon)
Festuca rubra L.: U. S. (Oregon)

Hordeum bulbosum L.: Russia, Syria, Turkestan, Turkey, Yugoslavia
Hordeum leporinum Link: Algeria, Australia, Iran, Spain, Turkey
Hordeum vulgare L.: Bulgaria, Iran, Iraq, Japan, Yugoslavia
Lolium multiflorum Lam.: U. S. (Idaho, New York)
Lolium perenne L.: U. S. (New York)
Lolium remotum Schrank: Denmark
Secale cereale L.: Austria, Czechoslovakia, France, Germany, Roumania, Switzerland, Turkey, U.S. (Idaho, Montana, New York, Oregon, Utah)
Triticum aestivum L.: Argentina, Austria, Canada, Czechoslovakia, Germany, Iran, Italy, Persia, Switzerland, U. S. (California, Colorado, Idaho, Indiana, Michigan, Montana, New York, Oregon, Utah, Washington), Yugoslavia
Triticum spelta L.: Germany

The synonymous relationship of the wheat dwarf bunt fungus to several other bunt fungi of various grasses and cereals in many parts of the world denies, in our opinion, the suggestion by Riehm (10) that dwarf bunt of wheat arose during the present century as a result of natural hybridization between races of common bunt, *Tilletia caries,* or as a mutant thereof. Instead, the present data are considered as evidence additional to that already presented in support of Conners' (2) hypothesis that dwarf bunt of wheat arose long ago from a similar smut on related grasses in the mountainous areas of Europe or the Caucasus range. We think that dwarf bunt of wheat is merely one part of a complex species which occurs on many grasses and cereals in many parts of North America, Asia, Europe, South America, Australia and North Africa, and which, in the past, has been designated under various specific epithets in the genus *Tilletia.*

Summary

Intensive critical study of hundreds of collections of *Tilletia* spp. on various grasses and cereals has uncovered several which are morphologically very similar to the organism causing dwarf bunt of wheat and many grasses and now recognized as *T. contraversa.*

Comparison of type material of *Tilletia pancicii* on *Hordeum* spp., and *T. calospora* on *Alopecurus* has led to the conclusion that these species should be reduced to synonymy under *T. contraversa.* Likewise, a comparison of many collections of *T. hordei* has led to the tentative conclusion that this species is also merely a synonym of *T. contraversa.* Final disposition of *T. hordei* must await a study of the type, not yet available.

These studies have incidentally revealed many new hosts of the complex species, *Tilletia contraversa.* These include species of *Aegilops, Agropyron, Alopecurus, Bromus, Elymus, Hordeum,* and

Lolium. These have been variously deposited in the herbaria as *T. caries, T. tritici, T. guyotiana, T. lolii, T. bornmülleri, T. calospora, T. hordei, T. pancicii,* and *T. brevifaciens.*

An emended description of *Tilletia contraversa,* as a composite species, is given, together with an up-to-date enumeration of the hosts and the geographic distribution. According to the concept of *T. contraversa* herein proposed, eight species and varieties are reduced to complete or partial synonymy under *T. contraversa.* The hosts of the composite species include *Aegilops* (3 species), *Alopecurus* (1 species), *Agropyron* (15 species), *Arrhenatherum* (1 species), *Bromus* (2 species), *Dactylis* (1 species), *Elymus* (4 species), *Festuca* (2 species), *Hordeum* (3 species, including cultivated barley), *Lolium* (3 species), *Secale* (1 species, *cereale),* and *Triticum* (2 species, *spelta* and *aestivum*). *T. contraversa,* in the emended sense, occurs in North and South America, Asia, North Africa, Australia, and Europe.

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SOME NEW DISTRIBUTION AND EARLY INCIDENCE RECORDS OF DWARF BUNT OF WHEAT AND RYE*

George W. Fischer and Ruben Duran†

Ever since dwarf bunt of wheat was first recognized as distinct from common bunt of wheat (17) there has been much conjecture both in the United States and elsewhere as to the origin of this smut. Fischer and Tyler (7) showed that according to available published data at the time, the earliest authentic record of dwarf bunt anywhere was 1931. Despite the record, it was generally believed that dwarf bunt had existed long before. Fischer and Tyler (*op. cit.*) urged a general restudy of herbarium specimens, especially where dwarf bunt of wheat is known to occur, to determine as closely as possible the dates and places of first incidence in other states and countries. Conners (1) pointed out the morphological similarity of dwarf bunt of wheat and grasses (to which Fischer (4) had given the name *Tilletia brevifaciens*) to Kühn's *T. contraversa,* on *Agropyron* spp. in Europe and western Asia. He suggested that wheat probably first contracted dwarf bunt from native species of *Agropyron* in the mountainous areas of Europe. He further believed that it was later introduced into North America either on wheat or on one of the cultivated species of the related genus *Agropyron.* If this hypothesis is correct, dwarf bunt of wheat probably occurred in Europe long before it was recognized as an entity different from common bunt of wheat. Riehm (12) considers it more plausible that dwarf bunt originated during the present century as a mutant from *T. caries* or by hybridization between races of this species.

Recently evidence was uncovered of earlier incidence of dwarf bunt of wheat than the hitherto established 1931. Lindquist (11) presented evidence, based on herbarium specimens, that the disease occurred in Argentina as early as 1915. This finding not only

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established an earlier date but also an entirely new locality for dwarf bunt of wheat. Tingey (13) presented circumstantial evidence that dwarf bunt occurred in New York state as early as 1923. He pointed out that the anomalous results Faris (3) obtained with a collection of *"Tilletia tritici"* from the Dawson variety of wheat certainly indicate that Faris was dealing with dwarf bunt rather than common bunt. Tingey (14) has also presented similar circumstantial evidence that dwarf bunt of wheat occurred in Michigan as early as 1892 and in Oregon in 1926. As noted by him (14, footnote 2) the suspected early incidence in Michigan was corroborated by I. L. Conners and R. L. Kiesling in a study of herbarium specimens preserved at Michigan State University. We were able to confirm the identification from parts of these collections sent by Dr. Kiesling.

Incidental to a taxonomic revision of the genus *Tilletia,* it has been possible for us to study hundreds of herbarium specimens of wheat bunt from all over the world. Any whose macroscopic appearance suggested dwarf bunt (smut balls tending to be hard and globose, thus spreading the palea and lemma more than is typical of common bunt) were given careful microscopic examination. Since it has been established that dwarf bunt occurs on rye (15, 16), every available specimen of bunt on rye was examined microscopically. As a result, many new records of geographic distribution, early incidence, or both, have been uncovered. A brief preliminary report on some of these has already been made (6). The present paper is a more detailed and more complete account of the results.

The data for the herbarium specimens that are considered to establish previously unrecognized records of dwarf bunt of wheat and rye are summarized in Table I. The spore characters for most of the collections are illustrated in Figures 1 and 2.

The earliest records of dwarf bunt incidence in the United States are now pushed back to at least 1860. Study of the Michigan collections in comparison with collections from a wide range of other locations, substantiates Tingey's (14) circumstantial evidence that dwarf bunt occurred in Michigan as early as 1892. Collection 571 (Table I and Fig. 1) even antedates this early record by two years.

Some interesting new records for New York state have been uncovered. Although no date was recorded for no. 212, it is believed to be around 1860-1890, or possibly even earlier. Concerning

TABLE I. COLLECTIONS ESTABLISHING NEW RECORDS OF EARLY INCIDENCE OR GEOGRAPHIC LOCATION FOR DWARF BUNT OF WHEAT AND RYE IN UNITED STATES, EUROPE AND ASIA

Acc. No.[a]	Host	Locality	Year	Collector	Where Deposited	Original Determination
			UNITED STATES			
212	Wheat	Herkimer Co., New York	Ca. 1860	C. F. Austin	N. Y. Bot. Garden ex Torrey Herb.	T. tritici
210	Rye	Syracuse, New York	1892	L. M. Underwood	N. Y. Bot. Garden ex Underwood Coll.	Ustilago on rye
571	Wheat	Oakland Co., Michigan	1890	M. B. Cooley	Herbarium-Mich. State University	T. tritici Low Smut
312	do	Batavia, N. Y.	1920	G. L. Zundel	U. S. Nat'l Fungus Collections	T. tritici
263	do	New Carlisle, Indiana	1917	G. A. Osner	Herb. Univ. Toronto ex Herb. H. S. Jackson	do
314	do	Jacksonville, California	1917	H. B. Humphrey	U. S. Nat'l Fungus Collections	do
304	do	Wayne Co., Utah	1918	G. L. Zundel	do	do
305	do	Rockland, Idaho	1918	G. L. Zundel	do	do
572	do	Pendleton, Oregon	1921	H. P. Barss	Oregon State College Herbarium	do
573	do	LaGrande, Oregon	1921	do	do	do
306	do	West Seneca Co., New York	1923	J. A. Faris	Brooklyn Bot. Garden	do
309	do	Seneca Co., New York	1923	do	do	do
			EUROPE AND ASIA			
507	Wheat	Velerlavin, Czechoslovakia	1847	J. Opiz	Herb. Czech. Nat'l Mus., Prague	Uredo sitophila a tritici
452	do	Preus-Munster, Germany	1858	Unknown	State Botanical Collections, Munich	Uredo sitophila
450	do	Schoeneberg, Germany	1878	Joseph Paul	do	T. caries
451	do	Salzburg, Austria	1884	von Niessl	do	T. caries
335	Rye	Ratibor,[b] Germany	1874	Unknown	State Museum of Natural History, Stockholm	T. secalis
396	Wheat	Schweidnitz,[c] Germany	1891	Schoepker	Roy. Bot. Garden, Edinburgh	T. tritici
130	Rye	Schoeneberg, Germany	1891	Joseph Paul	U. S. Nat'l Fungus Coll., Beltsville, Md.	T. caries
392	Wheat	Sultanabad, Iraq	1908	J. Bornmuller	State Museum of Natural History, Stockholm	T. caries
307	do	Belgrade, Yugoslavia	1944	V. Lindtner	Herbarium, University of Michigan	T. tritici[d]

[a] Authors' Accession number
[b] Now Raciborz, Poland
[c] Now Swidnica, Poland
[d] Issued in the exsiccati series: Lindtner, Herbarium Musei Historico-Naturalis Serbiae, Fasc. 1: Ustilaginales Jugoslaviae, No. 19

this particular accession, Dr. I. L. Conners advises:[1] "As collection no. 212 is ex Torrey Herb., it seems to me that the date is almost certain to be two or three decades before 1890 (the date we earlier arbitrarily assumed), for John Torrey lived from 1796 to 1873. Specimens are unlikely to have been added to his herbarium after his death. C. F. Austin (the collector in the case of accession no. 212) would appear to be Coe Finch Austin, 1831-1880, a botanist living at Closter, N.J." Very probably then, dwarf bunt of wheat has occurred in New York state for at least a century. Accession nos. 306 and 309, representing collections from Seneca County by J. A. Faris, certainly substantiate Tingey's (13) circumstantial evidence that dwarf bunt occurred in New York state as early as 1923. Of special interest is no. 210, representing dwarf bunt on rye. Made in 1892, near Syracuse, N.Y., by L. M. Underwood, who labeled it "Ustilago on Rye," it was finally deposited in the Herbarium of the New York Botanical Garden, along with the rest of the Underwood collections. H. S. Jackson (10) studied this collection in 1920, and reported that the collection is a *Tilletia*, with reticulations higher and wider than in typical *T. tritici* (*T. caries*). Comparing this New York collection with European exsiccati of *T. secalis* (Corda) Kühn, Jackson (*op. cit.*) considered that it should properly be referred to this species and that the collection in question constituted, therefore, the first report of the rye bunt from North America. In the present study, the spores of this Underwood collection were easily observed to correspond well with wheat dwarf bunt collections from the same state (Fig. 1) and elsewhere, further declaring the existence of dwarf bunt in upper New York state in the early 1890's.

Dwarf bunt has not been reported previously from Indiana. However, accession no. 263, representing a collection made by G. A. Osner in 1917, near New Carlisle, leaves little doubt that it was there then.[2] The record is not surprising, considering that the Indiana location is only about 20 miles from Buchanan, Michigan, where dwarf bunt was collected in 1892 (14).

Tingey believes that dwarf bunt occurred in Utah as early as 1903. He states (14) "there is no doubt about it being in Utah in the early 1920's," and that "dwarf bunt was already serious in

[1] In correspondence.

[2] We are indebted to I. L. Conners for calling this collection to our attention.

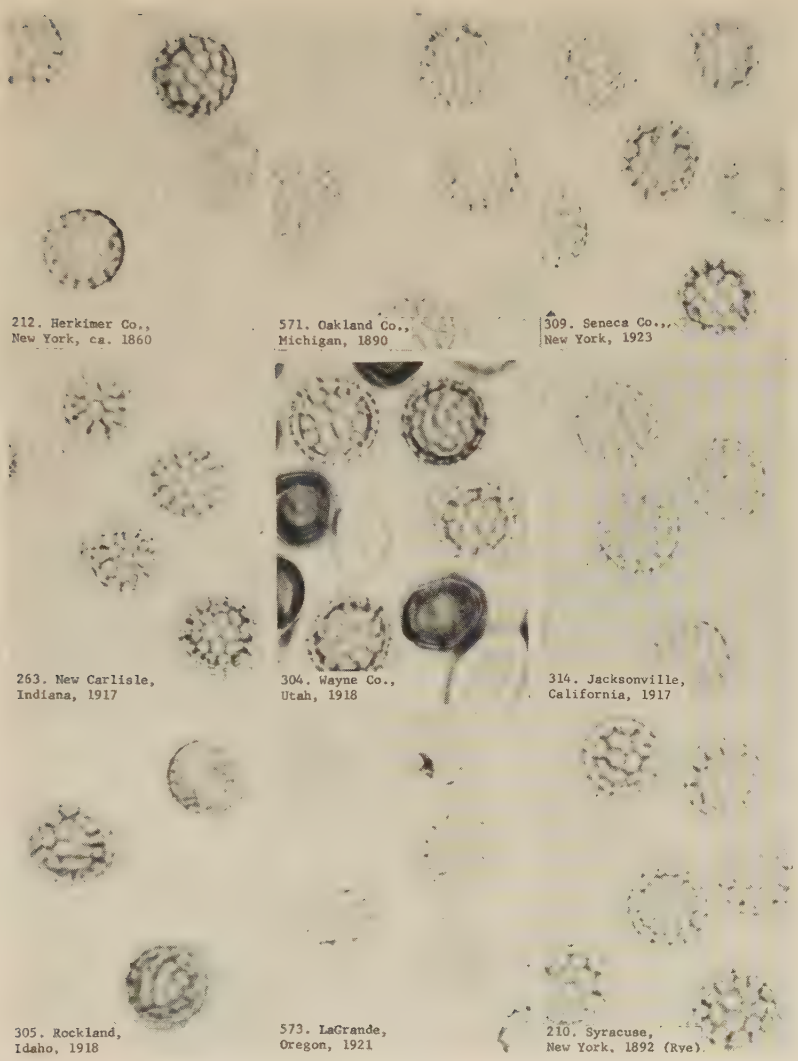

Figure 1. Photomicrographs of spores from herbarium specimens eestablishing records of distribution or early incidence of dwarf bunt of wheat and rye in the United States. See Table I for collection data. Somewhat retouched to attain perspective. × approx. 600.

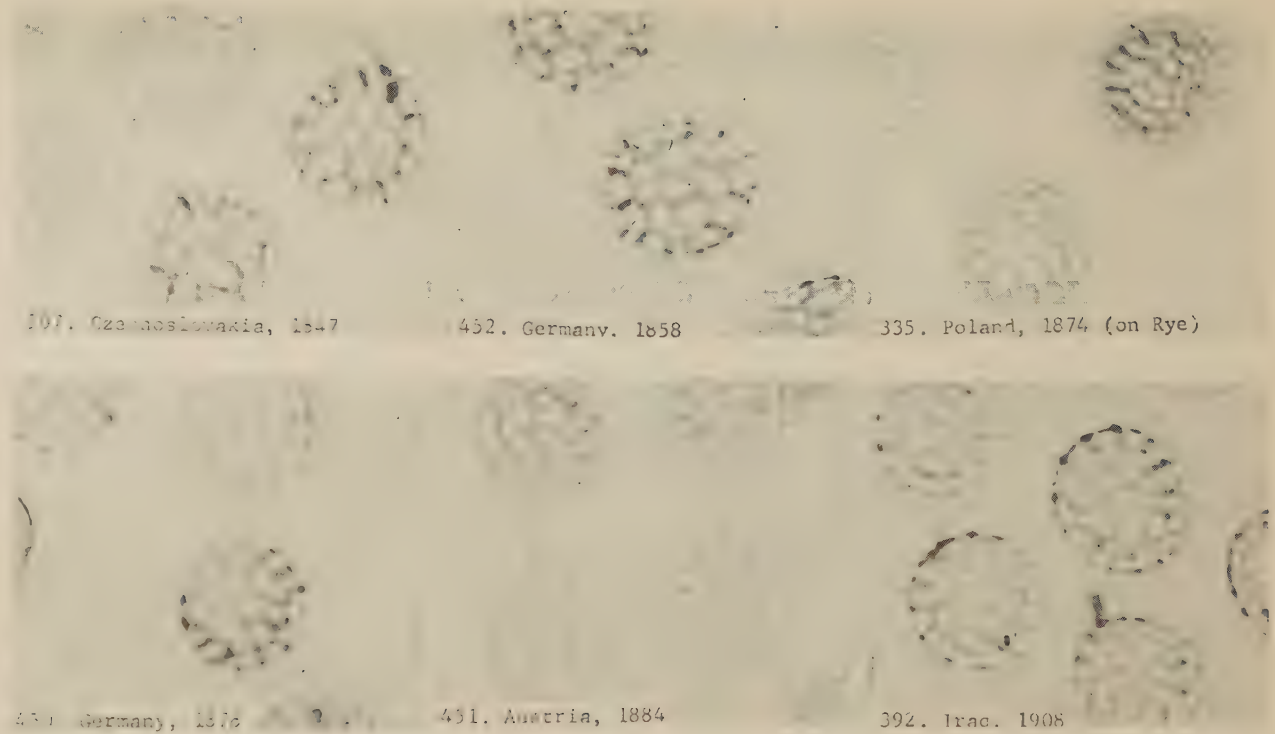

Figure 2. Photomicrographs of spores from herbarium specimens establishing records of early incidence of dwarf bunt of wheat and rye in Europe and Asia. See Table I for collection data. Somewhat retouched to attain perspective × approx. 600.

northern Utah and southern Idaho as early as 1925. . . ." So far as we are aware, these statements have not hitherto been substantiated by comparative study of representative collections. However, our accessions 304 and 305, representing collections in Wayne County, Utah, and Rockland, Idaho, in 1918 by G. L. Zundel, are definitely dwarf bunt, substantiating Tingey's claims of early existence in those states. The Wayne County collection also contained the smooth-spore bunt caused by *Tilletia foetida* (Wallr.) Liro, as shown in Figure 1.

Likewise, Tingey (*op. cit.*) states "There is little doubt but that dwarf bunt was present in Oregon before 1927." Here again, a deduction is borne out by the present study. Accessions 572 and 573 represent collections made in 1921 by H. P. Barss in northeastern Oregon, an area where dwarf bunt is still a serious problem, not only on wheat, but also on cultivated grasses (8,9).

The records from Europe and Asia given in Table I and based on collections illustrated in Figure 2 establish earlier incidence of dwarf bunt for this continent than for North America. Of particular interest in this regard is accession 507, representing a collection made in 1847 by J. Opiz in what is now Czechoslovakia. So far as we have been able to determine, dwarf bunt has not been considered a problem in Czechoslovakia. Yet the spores of Opiz' collection (Fig. 1) leave little doubt of its existence there at least as early as 1847. The antiquity of the collection is further borne out by the identification on the packet: "*Uredo sitophila* α *tritici* Opiz."

Table I also shows several collections which seem to declare very early incidence of dwarf bunt in Germany, where previously no earlier record than 1931 had been established (7). Rivaling the 1847 record in Czechoslovakia is accession 452, based on a collection of "*Uredo sitophila*" in Germany,[3] dated 1858. Less spectacular but still nearly half a century earlier than previously established records of incidence in Germany, Austria, and Poland are nos. 450-452, 396, 335, and 130. The latter two indicate also that dwarf bunt occurred on rye in Poland as early as 1874 and in Germany as early as 1891.[4] These very early records of dwarf bunt of wheat

[3] The script on the packet was not very legible. The locality appeared to be "Prems-münster," but Dr. L. J. Tyler suggests (in correspondence) that the place is Preus-Münster, or a monastery in Prussia.

[4] Dwarf bunt is considered to be one of three reticulate-spore species of *Tilletia* occurring on rye and which in the past usually have been given the convenient designation of *T. secalis* (Corda) Kühn (5).

and rye in Europe have additional interest when one considers that only in the last two decades has the disease been recognized as a problem in Europe. Riehm (12) has recently reviewed the literature on this subject.

Accession 307, based on a collection of *"Tilletia tritici"* near Belgrade in 1944 clearly indicates by the appearance of the spores (Fig. 2) that dwarf bunt is present in Yugoslavia, though apparently not hitherto recognized.

Likewise, we know of no report of dwarf bunt of wheat or rye from Iraq. Yet the appearance of the spores of accession 392 (Fig. 2) strongly indicates that Bornmüller collected dwarf bunt of wheat near Sultanabad nearly half a century ago.

The similarity of the spores of these collections of bunt on wheat and rye from North America, Europe and Asia, as shown in Figures 1 and 2, indicates rather strongly that all the collections from all three continents represent the same species. It is readily admitted that perhaps there is some hazard in assigning all these to dwarf bunt solely on the basis of the gross appearance of the smutted spikes and the microscopic appearance of the spores. It might be argued that this procedure takes too much for granted in assuming that these collections were taken from dwarfed plants. Nevertheless, herbarium specimens of wheat and rye bunts rarely consist of more than the bunted spikes. Since the spores of dwarf bunt have distinct characteristics, which are recognized by many investigators, we think we have taken comparatively little for granted in asserting that the records herein presented do pertain to dwarf bunt of wheat and rye. The officials of herbaria have been very cooperative in complying with our requests for specimens. Even so, there are undoubtedly many hundreds of old collections of bunts of wheat and rye in Europe and Asia which would still further extend the knowledge of the early incidence and distribution of dwarf bunt. We hope our colleagues in Europe and Asia will make such a study of the collections in their countries.

Thus far, it seems fairly certain that dwarf bunt of wheat and rye occurred in the United States (New York) as far back as 1860, in Europe (Czechoslovakia, Germany, Austria, and Poland) as early as the middle of the last century, and in Asia Minor as far back as 1908. These records, together with data on dwarf bunt of barley and grasses (2), make it seem unlikely that dwarf bunt originated during the present century as a hybrid between races of *Tilletia*

caries or as a mutant of that species, as suggested by Riehm (12). Instead, Conners' hypothesis (1) that dwarf bunt of wheat arose long ago from a similar smut on related grasses in the mountainous areas of Europe or the Caucasus range now seems well substantiated.

Summary

In a study of hundreds of herbarium specimens of *Tilletia* spp. on wheat and rye from all over the world the following new records of early incidence and geographic distribution have been established for dwarf bunt of wheat and rye, *T. contraversa* Kühn (*T. brevifaciens* G. W. Fisch.): U.S.A., on wheat, New York: Herkimer Co., *ca.* 1860; Batavia, Genesee County, 1920; Seneca Co. 1923; Michigan: Oakland Co., 1890; Indiana: New Carlisle, 1917; California: Jacksonville, 1917; Utah: Wayne Co., 1918; Idaho: Rockland, 1918; Oregon: Pendleton and LaGrande, 1921. On rye: New York: Syracuse, Onondaga County, 1892. Europe, on wheat: Czechoslovakia, 1847; Germany, 1858-1878; Austria, 1884; Yugoslavia, 1944. Asia: Iraq, 1908. On rye: Poland, 1874 and Germany, 1891.

These records extend our knowledge of the existence of dwarf bunt of wheat and rye more than a century farther back than the previously established 1931. The specimens studied had been determined previously as various species of *Tilletia, Ustilago,* and *Uredo.* Some of these very early records from Europe are from the same regions where *T. contraversa* has long been known to occur on *Agropyron* spp. This fact seems to substantiate Conners' suggestion that wheat (and rye) first contracted dwarf bunt in the mountainous areas of Europe where *T. contraversa* occurs naturally on native species of *Agropyron* and that dwarf bunt was subsequently introduced into North America.

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THE SPECIES OF *TILLETIA* ON RYE*

George W. Fischer†

During the course of a critical taxonomic review of the described species of the genus *Tilletia,* it became evident that there has long been considerable confusion as to the identity of and distinction among the species that occur on rye *(Secale cereale* L.). The comparative morphology and physiology of these species has been variously interpreted, thus giving rise to conflicting conclusions and opinions on their identity. Likewise, there are a great many conflicting records on the occurrence, distribution, pathogenicity, physiology, and symptomology of the bunt fungi *(Tilletia* spp.) on rye.

The confusion and misunderstanding concerns those species of *Tilletia* on rye that have reticulate spores. These species have been variously designated as *Tilletia secalis* (Corda) Körn., *T. secalis* Körn., *T. secalis* (Corda) Kühn, *T. secalis* Kühn, *T. contraversa* Kühn, *T. brevifaciens* G. W. Fisch., *T. caries* (DC.) Tul., *T. tritici* (Bjerk.) Wint., and *T. tritici* f. sp. *secalis* Pichler. The principal purpose of this paper is to try to clarify some of this confusion.

Smooth-spored Species

Tilletia foetida (Wallr.) Liro *(T. levis* Kühn) is the only smooth-spored species of *Tilletia* attacking rye, and reports of such incidence have been few. Apparently the first is that of Johnston (25) in 1927 in Kansas. In a severely smutted field of wheat with an admixture of rye, three out of several thousand heads of the rye were found with bunt infection caused by *T. foetida.* Similar incidence of this species on rye has been recorded by Lobik (35) in the North Caucasus in 1930. Lobik *(op. cit.)* later successfully inoculated wheat with the *T. foetida* spores from rye. Ivanoff (27) reported the incidence of *T. foetida* on rye in Bulgaria, and Hirschhorn (26) has described the occurrence of *T. foetida* and what appeared to be hybrids between this species and *T. caries* on rye in Argentina.

These isolated records of the spontaneous incidence of *Tilletia*

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foetida on rye would indicate only slight susceptibility of this host. This conclusion is borne out by the limited success of inoculation experiments. Bressman (5), after several attempts, was finally able to infect two varieties of rye, Rosen and "Oregon Sel. 1." Nieves (42), working in Argentina, found that one of two physiologic races of *T. foetida* was able to infect rye. Later, however, Nieves (43) found that all of the four races of *T. foetida* that he was able to distinguish in Argentina were able to infect rye, as represented by the variety Petkus, although the infection percentages were very low.

T. foetida being the only smooth-spored species of *Tilletia* attacking rye and other cereals, there has been no problem of confused identity with other species.

Reticulate-spored Species

The first of the reticulate-spored species of *Tilletia* to be recognized on rye was *T. secalis*. This was originally described as *Uredo secalis* by Corda (10) in 1848,[1] who found it in Bohemia. Rabenhorst (46) in the following year described a *Uredo secales* as "der eigentliche Roggenbrand, eine neue Pilzart." In doing so, Rabenhorst cast considerable suspicion on the validity of Corda's *Uredo secales* as applying to any *Tilletia* sp.: "Endlich Herr Corda (Hlubek's Oekonomische Neuigkeiten 1846 Nro. 82) will den vermeinten Rausch- oder Roggenbrand als *Cladosporium herbarum* Lk. erkannt haben und fügt noch einem rothen Kornbrand hinzu." Further with regard to Corda's paper of the year before, Rabenhorst *(op. cit.)* comments:

> Was nun die Mittheilung Corda's in den oben genannten Zeitschrift betrifft, so weiss ich sie nicht zu deuten; den an den aus Corda's eigner Hand erhalten, mit seinen oben angeführten Pilzen angeblich inficirten Roggenahren fand sich weder ein *Cladosporium* noch ein *Septosporium,* sondern meine *Ustilago* im vollkommen ausgebildeten Zustande.

Rabenhorst's *(op. cit.)* description of the micro- and macromorphology of *Uredo secales* (which he declared to be "keine Rubigo, sondern eine *Ustilago*") makes it doubtful that he was dealing with any *Tilletia* sp. but an *Ustilago* and more than likely *U. nuda*. However, the immediately pertinent feature of Rabenhorst's ac-

[1] Liro (34) cites several much earlier records (1596-1768) of the occurrence of bunt on rye, under such names as *Ustilago secalina, U. secalis,* and *U. secalena,* but Volkart (53) believes that at least some of these very early records pertain to ergot, *Claviceps purpurea,* and to loose smut, *Ustilago nuda* (Jensen) Rostr.

count of *Uredo secales* is the above-quoted comments on Corda's paper. It is difficult to know how to interpret these comments. Possibly Corda, besides giving an account of a bunt and giving it the name *Uredo secalis* (rusts and smuts alike were commonly placed in *Uredo* in Corda's day), also mentioned another disease, which he wished to ascribe to *Cladosporium herbarum,* and when he sent specimens to Rabenhorst the latter recognized the fungus as a smut of the *Ustilago* type. On the other hand, Rabenhorst's comments could be interpreted as nullifying Corda's *Uredo secalis* as not applying to a *Tilletia* at all. This possible interpretation is supported by the fact that when Kühn (33) in 1876 described "*Tilletia secalis,* eine Kornbrandform des Roggens," he treated his fungus as a new species and did not designate *T. secalis* as a new combination with Corda's *Uredo secalis,* i.e., *Tilletia secalis* (Corda) Kühn. Kühn knew about Corda's binomial, for he stated that the appearance of rye smut had not been reported since Corda reported it in 1848 and Rabenhorst in 1849. On the other hand, Kühn stated flatly that Rabenhorst's *U. secales* is not a *Tilletia* and is quite distinct from "*T. secalis* mihi," but made no comments on the relationships or identity of Corda's *Uredo secalis.*

Körnicke (31) recognized Rabenhorst's *Uredo secales* as a *Tilletia* and assumed the same for Corda's *U. secalis.* In 1872 he made the combination *Tilletia secalis* on the basis of Klotzsch's No. 1399 in his exsiccati series, "Herbarum Vivum Mycologicum," issued as *Uredo secales* Rabenh. In the present study, examination of this exsiccatum confirms that it is indeed a *Tilletia.* Since Klotzsch's No. 1399 is probably type material of Rabenhorst's *Uredo secales* (collected by Rabenhorst in 1847), one might wonder at Kühn's *(op. cit.)* categorical statement that Rabenhorst's *U. secales* is *not* a *Tilletia,* if it were not for the fact that, as already stated, Rabenhorst's (46) own account of his *Uredo secales* makes it practically certain that he was describing a species of *Ustilago* and not a *Tilletia.* It is very difficult to reconcile these conflicting records unless we assume that Rabenhorst and Corda were both dealing with mixed collections of *Ustilago* and *Tilletia.*

This confused and uncertain background of *Tilletia secalis* is largely responsible for the different authorities credited with the binomial. Apparently there is no type material of Corda's *Uredo secalis.* The type material of Rabenhorst's *Uredo* (Ustilago) *secales* is obviously a *Tilletia* (see Fig. 1) although his published descrip-

tion clearly refers to a *Ustilago*. Rabenhorst states that he does not know how to interpret Corda's report (10) because in material of Corda's fungus which Corda himself sent to Rabenhorst the latter found neither a *Cladosporium* nor a *Septosporium*, but his (Rabenhorst's) *Ustilago* in fully developed condition. Kühn declared that Rabenhorst's fungus is not a *Tilletia* and proceeded to (apparently) describe the *Tilletia* on rye as new, under the binomial *T. secalis* Kühn. However, if Körnicke (31) was actually studying Corda's *Uredo secalis* as well as Rabenhorst's *Uredo secales* when he placed the fungus in the genus *Tilletia*, then Körnicke's combination must stand.

Regardless of who gets priority in the naming of *Tilletia secalis*, what is alleged to be the same fungus has, since Corda's report from Bohemia in 1848, been reported from many different localities, and sometimes in severe epiphytotic form. Von Niessl (41) in 1876 reported that in every rye field he could observe in the vicinity of Brünn he found heavy infections of rye bunt. He believed it had been introduced much earlier but had gone unnoticed until the epiphytotic of 1876. Sigriansky (50) in 1925 stated that *T. secalis* was becoming dangerously prevalent in some districts of European Russia, sometimes taking 45 per cent or more of the crop. Jaczewski (29) likewise reported on the alarming development of *T. secalis* in European Russia. The severity fluctuated considerably from 1884, when the rye bunt was first recorded in Russia, to 1921 which was the last included year in Jaczewski's report. The worst of these years was 1910, when about 90 per cent of the crop was destroyed in the government of Podolia in southwest Russia. In 1925 Davydoff (11) reported *T. secalis* from Siberia. Bubak (6) reported an epiphytotic development of this rye bunt in Bohemia in 1910, with 50 per cent of the rye heads destroyed over a large area around Pacov.

Besides the epiphytotics mentioned above, there have been numerous other records of the incidence of "*Tilletia secalis*" on a smaller scale: Russia [Buchheim (7), Morozov (38), Sigriansky (50)]; Austria [Pichler (44), Niemann (39)]; Finland [Karsten (30)]; Germany [Kühn (32)]; Czechoslovakia [Blattny (4)]; Bulgaria [Malkov (36)]; Turkey [Niemann (39)]; Roumania [Savulescu (47)]; Italy [Schroeter (49)]; France [Viennot-Bourgin and Ziegle (52)]; Britain [Ainsworth and Sampson (2)]; and the United States [Jackson (28)].

Irrespective of conflicting authorities for *Tilletia secalis,* it was considered a good distinct species until the advent of reports of the spontaneous incidence of common bunt of wheat *(T. caries)* on rye and the subsequent demonstrations of the susceptibility of rye by inoculation experiments. In 1922 Gaines and Stevenson (16) reported the occurrence of *T. caries* on rye × wheat and wheat × rye hybrids on the College Farm, State College of Washington, and in 1923 the same authors (17) reported the same bunt on rye itself as well as on the hybrids between wheat and rye. They found bunt on one plant of common rye, one F_2 plant of a rye × wheat hybrid and two F_2 plants of a wheat × rye hybrid. The spore characters and the fetid odor convinced the authors that the bunt was due to *T. caries.* They followed up these observations with inoculations of nine varieties of winter rye, 14 varieties of spring rye and the F_3 generation of each of four rye × wheat hybrids. A light infection resulted on four of the winter rye varieties and on only one of the spring ryes. Much more infection resulted on the hybrid material. Gaines and Stevenson *(op. cit.)* suggested that perhaps *T. secalis* and *T. tritici (T. caries)* are in reality the same fungus parasitizing different hosts.

Hirschhorn (26) likewise thought that *Tilletia secalis* and *T. caries* are poorly differentiated. Schellenberg (48) stated flatly that *T. secalis* is identical with *T. tritici (T. caries)* and cited Appel as authority that the latter smut fungus will infect rye and barley.

Besides Gaines and Stevenson (17), several others have succeeded in infecting rye with wheat bunt caused by *Tilletia caries.* According to Schellenberg *(op. cit.),* Appel (3) in 1909 was the first, thus 14 years ahead of Gaines and Stevenson *(op. cit.).* Ducomet (13) tried to infect rye with *T. caries* for several years, finally succeeding in 1926. He also raised the question of *T. caries* and *T. secalis* possibly being specialized forms of the same morphological species. Dillon Weston (12) in 1928-29 inoculated 24 "samples" of rye with *T. caries* from different varieties of wheat and obtained infection "in some cases," but not more than 2 per cent in any case. He repeated the experiment the following year using spores from the rye infection of the previous year as well as from wheat. He obtained light infection of the rye in both cases, but no higher percentage on rye inoculated with spores from rye.

Bressman (5) in 1931 reported on inoculation experiments of rye with bunt of wheat. Using 16 collections of bunt as inoculum

(presumably representing both *T. caries* and *T. foetida)* selected from 100 collections from American and foreign sources, Bressman *(op. cit.)* was unable to infect spring rye. He then selected 26 "outstanding" collections of wheat bunt and used them to inoculate Oregon Rye Sel. 1 and five collections on Rosen rye. Only one collection (designated as race 9) infected rye, producing 6.1 per cent in Oregon Sel. 1 and 3.5 per cent in the Rosen rye. It was noted that in the Oregon Sel. 1, normally showing considerable sterility so that many of the florets do not produce seed, the smutted spikes bore bunt balls in practically all of the florets. The spores of this race of *T. caries* that attacked rye "averaged about 19μ in diameter and were typical of *Tilletia tritici" (T. caries).*

Nieves (42) likewise was able to infect rye with two races of *T. caries,* but obtained very low percentages of infection. In a second series of experiments Nieves (43) found that three out of nine physiologic races of *T. caries* were able to infect Petkus rye. Again, the infection percentages were very low, not exceeding 1 per cent. Viennot-Bourgin (51) in 1929 and again in 1934 obtained very slight infection of rye with *T. caries* by seed inoculation and planting in autumn. He concluded that infection of rye by wheat bunt is always exceptional and must be considered in the same light as the occasional incidence of *T. caries* on wild grasses. Viennot-Bourgin volunteered the opinion that *T. caries* and *T. secalis* are not to be considered the same species.

Volkart (53) also took exception to the view that *Tilletia secalis* and *T. caries* are of the same species. He emphatically stated that assumed identity has no basis in the fact that, under heavy inoculation, *T. caries* will infect rye. Volkart *(op. cit.)* considered *T. secalis* distinct on the basis of (1) greatly irregular severity from year to year; (2) no dwarfing of the infected culms, the latter standing more erect and taller than the healthy ones; (3) spore morphology *(T. secalis* spores larger, more deeply reticulate); (4) spore germination (extremely difficult to induce), and (5) infection factors (obviously different from *T. caries).*

Among the more recent studies of rye bunt are those of Pichler (44). A severe development of this smut in 1946 in Austria (vicinity of Rauris) apparently precipitated an investigation of it. Using spores of the rye bunt from Rauris and also spores of wheat bunt[2] from the same locality Pichler *(op. cit.)* inoculated seven

[2] Species not stated; indicated only as "Weizensteinbrandsporen."

varieties of winter rye and eight of winter wheat by the usual method of dusting the seed with spores. Infection resulted in all of the rye varieties inoculated either with spores of the rye bunt or with spores of the wheat bunt, although the percentages of infection were low in all cases. The inoculations of rye with rye bunt spores resulted in 1.7-5.5 per cent infection, and from the wheat bunt spores 0.1-2.8 per cent. Where winter wheats were inoculated with the same spore material, four of the eight varieties showed no infection at all from rye bunt, and there was only 0.1-0.4 per cent in the other four varieties. However, the percentages of infection were likewise low where the wheats had been inoculated with spores from wheat: 0.4-2.9.

Pichler justly questions the validity of the slight infection of wheat with rye bunt spores because the inoculated seed had not had the usual prior disinfection treatment, even though the seed had come "aus einem brandfreien Bestand." He also pointed out that in recent years smut appeared in stands even where the seed had been treated with 0.1 per cent formaldehyde for 30 minutes. In a more extensive series of inoculations, in 1947, using rye bunt spores and wheat bunt spores from various sources, he was completely unsuccessful in attempts to infect wheat.

The already confused situation with respect to the relationship of *"Tilletia secalis"* to *T. caries* has become a bit more confused in recent years with reports from several sources that apparently dwarf bunt of wheat and grasses, *T. contraversa* Kühn[3], had been found on rye. In 1952 Warmbrunn (54) reported the occurrence of dwarf bunt on rye in Germany, causing conspicuous dwarfing but without the excess tillering usually produced on wheat. He was certain it was dwarf bunt because of the morphology of the spores, and he emphasized that the smut could not be considered a dwarfed type of *"T. secalis."* In 1952 dwarf bunt was found in rye in Utah (55), in 1954 (23) in Oregon, and in 1956 in Idaho and Montana (24). In each case there was the characteristic dwarfing. Viennot-Bourgin and Ziegle (52) reported an infestation of *"Tilletia secalis"* in rye in France, stated to be the first report of this bunt fungus from that country. However, these authors state that there was about 40 per cent dwarfing (i.e., infested plants

[3] Also described as *Tilletia tritici nanifica* Wagner and *T. brevifaciens* G. W. Fisch., but now believed to be the old long-established species, *T. contraversa* (9, 14, 15).

40 per cent normal height), the spores were larger and with more pronounced reticulations than in *T. caries,* and that they showed similarity to *T. contraversa* which is abundant in the same area. These facts would seem to leave little doubt that these authors were dealing with dwarf bunt in rye.

These reports of the spontaneous occurrence of dwarf bunt in rye have been substantiated by infection experiments. Niemann (39) was able to infect rye with dwarf bunt from *Agropyron* sp., and Meiners (37) has infected wheat with dwarf bunt from rye. In contrast to these results, however, Aebi (1) obtained only negative results in cross-inoculation experiments with dwarf bunt from wheat and rye. Niemann *(op. cit.)* likewise was unsuccessful in reciprocal cross-infection experiments.

There are conflicting reports on the comparative physiology of *"Tilletia secalis," T. caries* and *T. contraversa.* Gassner and Niemann (18) showed that both dwarf bunt of wheat and rye bunt have very low infection temperatures (2° C.) as contrasted with 12° C. for common bunt of wheat. The species identity of this rye bunt is again uncertain, inasmuch as the authors considered it as *"T. secalis,"* with *T. caries* and *T. tritici* f. sp. *secalis* as synonyms. At least they did not regard this rye bunt as dwarf bunt. Gassner and Niemann (19) and also Pichler (45) likewise showed that dwarf bunt and rye bunt both require low temperatures for spore germination, but here again one cannot be sure whether these authors were studying the same organism others had studied under the name of *"T. secalis"* or were dealing with dwarf bunt *(T. contraversa)* on rye. The same problem of uncertain identity applies to a more recent study by Gassner and Niemann (21) wherein they showed that spore germination of the rye bunt fungus was strongly stimulated by heat sterilized soil decoction, whereas this substance inhibited spore germination in common bunt *(T. caries)* and dwarf bunt *(T. contraversa)* of wheat.

Buchheim (7) much earlier (1926) had stated that rye bunt will not infect wheat and that it *(T. secalis)* has different temperature and moisture requirements for spore germination than is true of *T. caries.* In 1954 Niemann (40) concluded that factors controlling spore germination and infection of the host are different in *T. contraversa, T. caries* and *"T. secalis."* For example, *T. secalis* spores germinated readily and abundantly in either light or total

darkness when on a heat-sterilized soil decoction medium, but *T. contraversa* did not.

Since dwarfing of the host is a pronounced symptom produced by the dwarf bunt fungus, it seems some clue to the possible relationship between dwarf bunt and rye bunt *(T. secalis)* might lie in the degree of expression of this symptom. This is not the case, however. While Warmbrunn (54), Gassner and Niemann (20), Niemann (39), and Viennot-Bourgin and Ziegle (52) all report dwarfing by *"Tilletia secalis,"* Rabenhorst (46), von Niessl (41), Cohn (8), Bubak (6), Volkart (53) and Pichler (44) either made no mention of dwarfing or they stated that *T. secalis* does *not* cause dwarfing of the host plants. Volkart *(op. cit.)* even stated that the infected heads stand *higher* than the normal. In the case where Pichler *(op. cit.)* reported absolutely no dwarfing of the host by rye bunt (which he designated as *T. tritici* f. sp. *secalis),* Niemann (37), allegedly using Pichler's (*op. cit.*) same spore material to inoculate Petkuser winter rye, obtained infection in which the bunted plants were, on the average, about 50 per cent dwarfed.

Gassner and Niemann (20) working with rye bunt, which they designated as *"T. secalis* Kcke. = *T. tritici* f. sp. *secalis* Pichler," noted that the infected rye culms were about 53 per cent dwarfed. A further interesting observation was the fact that even on partially infected plants the bunted culms were dwarfed but the healthy ones were of normal height. Since Gassner and Niemann *(op. cit.)* were using rye bunt that was at least supposed to be from the same source as that used by Pichler *(op. cit.)* wherein no dwarfing had resulted, they wondered if the dwarfing effect depends not only on the parasite but also on other (environmental) factors. They suggested that the difference in the results obtained with the same inoculum might be due to differences in climatic factors in the two areas concerned or to the use of different rye varieties.

Gassner and Niemann *(op. cit.)* expressed the opinion that a separation of dwarf and rye bunt as distinct species only on the basis of the dwarfing induced by dwarf bunt is indefensible, as long as there are no supporting morphological characters. They argued that the culm dwarfing is not a characteristic of the dwarf bunt fungus alone but is influenced by other factors (reaction of the host plant, environmental factors, etc.). From this we may infer that these authors are inclined to the view that the rye bunt of earlier workers is the same as dwarf bunt. Warmbrunn (54), on the other

hand, believed that the rye bunt he studied was merely wheat dwarf bunt transferred to rye and a different smut from the bunt described on rye by earlier authors.

The current confusion concerning the identity and relationships of the *Tilletia* spp. occurring on rye should be apparent from the foregoing review of the conflicting records, descriptions and observations in the literature. Niemann *(op. cit.)* concluded that it is uncertain whether different species of bunt fungi occur on rye or whether all observations on rye bunt concern the same species.

It is my belief that the existing confusion concerning the rye bunts has several contributing circumstances: (1) there are four distinct species of *Tilletia* occurring on rye *(T. foetida, T. caries, T. contraversa, T. secalis);* (2) some workers have concluded that, since rye is more or less susceptible to *T. caries, "T. secalis"* is probably merely *T. caries* on rye; (3) some workers actually have been concerned with *T. caries* in their studies of *"T. secalis"*, some with *T. contraversa,* and some with the true *T. secalis;* (4) there has been a tendency to designate any reticulate-spored bunt on rye as *"T. secalis,"* and (5) some workers unknowingly have used *admixtures of these different species as inoculum* since apparently in no instance has any attempt been made to use pure lines, relying instead on gross field collections. On the basis of a study of the comparative morphology of many collections of rye bunts from various parts of the world (mostly Europe, Asia and North America) the following attempt to differentiate these species is presented.

Key to the Species of Tilletia on Rye

1. Teliospores with smooth exospore ..... *T. foetida*
1. Teliospores with reticulate exospore 2
2. Areolae shallow (0.5-1.5μ deep) ..... *T. caries*
2. Areolae deep (1.5-3μ deep) 3
3. Bunt balls globoid, greenish black, more or less indurate, infected plants more or less dwarfed; spores encased in a hyaline gelatinoid sheath extending as much as 4μ (usually 1-2.5μ) beyond exospore ..... *T. contraversa*
3. Bunt balls elongate, brownish, fragile; infected plants not dwarfed; spores with little, or more often no sheath ..... *T. secalis*

Tilletia foetida (Wallr.) Liro

Tilletia foetida is the only one of the rye bunts with smooth spores. Since recognition of this species on rye has never been a problem, there is no need to discuss its morphology here.

Tilletia caries (DC.) Tul.

Of the scores of collections of rye bunts included in the present study, only four are here considered to belong to *Tilletia caries*. These were all made in the vicinity of Pullman, Washington in 1922 and 1923 by E. F. Gaines, and are represented by one collection included in Figure 1A. In these collections the bunt balls are rounded, comparatively small, and rather hard. The spores are quite regularly globose, pale grayish brown and are shallowly reticulate. The areolae are mostly 1.5-3.5μ in diameter and only about 0.5-1μ deep.

Tilletia caries on rye should be easily distinguishable from the next two species by virtue of the comparatively shallow areolae on the teliospores.

Tilletia contraversa Kühn

Like *Tilletia caries, T. contraversa* on rye is characterized by short, rounded smut balls. These are greenish black and are quite firm. The teliospores, however, are much more ornate, having a prominently reticulate exospore, encased in a hyaline, gelatinoid sheath that sometimes extends as much as 3-3.5μ beyond the reticulations (Fig. 1, G-I). Stunting of the host is also characteristic of *T. contraversa* on rye.

Collections of rye bunt included in the present study and here considered unquestionably to be dwarf bunt, *Tilletia contraversa,* have come from Austria, Bavaria, Germany, Turkey, and the United States (Idaho, Montana, New York, Oregon, Utah).

Tilletia secalis (Corda) Körnicke

As discussed earlier in this paper, it is difficult, if not impossible, to know for sure what organism the binomial *Tilletia secalis* refers to. Apparently Körnicke (32) considered that Kühn's *T. secalis* of 1876 is the same as his own of 1872 (31). Since Körnicke (32) stated that he recognized Corda's *Uredo secalis* as a species of *Tilletia* it might be assumed that Körnicke made a new combination from Corda's binomial. Since there seems to be no existing type of Corda's *Uredo secalis* and since Körnicke (31) was the first to

recognize that the rye smut is a *Tilletia,* it would appear that the acceptable binomial by the International Code of Botanical Nomenclature is *T. secalis* (Corda) Körnicke. Furthermore, since Körnicke recognized both Rabenhorst's *Uredo secales* and Corda's *Uredo secalis* as belonging to *Tilletia,* and since he based his *T. secalis* on the type material of Rabenhorst's *U. secales,* it might be assumed that the latter, therefore, is the type of *T. secalis.* In the absence of type material of Corda's *Uredo secalis* which would logically be the type of *T. secalis,* it becomes necessary to designate a lectotype for *T. secalis.* For this purpose, I propose the type of Rabenhorst's *Uredo secales,* issued as No. 1399 in Klotzsch's Herbarum Vivum Mycologicum. Through the courtesy of the Rijksherbarium, Leiden, this material was available for study, and is illustrated in Figure 1B.

Most of the rye bunt collections from Europe and Asia that have been included in the present study are considered to be *Tilletia secalis* as represented in Figure 1, B-F. As compared with *T. caries* and *T. contraversa, T. secalis* is characterized by (1) elongate, fragile bunt balls; (2) brown or reddish brown hue to the spore mass; (3) spores with narrower (0.5-1.5μ) areolae, 1.5-2.5μ deep,

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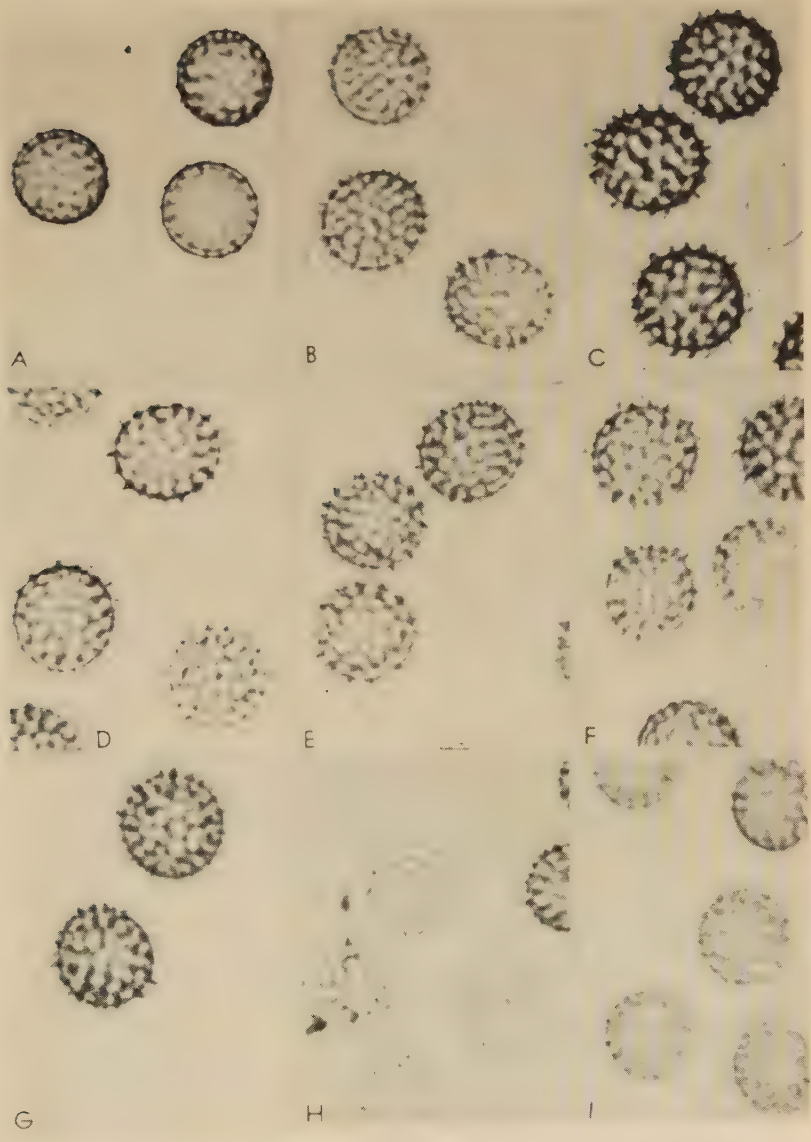
Figure 1. The reticulate spored species of *Tilletia* on rye. (A) *T. caries,* as represented by a collection made near Pullman, Washington by E. F. Gaines, 1923. (B-F) Common rye bunt, *T. secalis*: (B) "*Uredo secales* Rabenh.", Type, issued as Klotsch's Herbarum Vivum Mycologicum No. 1399; (C) "*T. secalis* Jul. Kuhn", Type, issued as Rabenhorst's, Fungi Europaei No. 2191; (D) Bubak's Kryptogamae exsiccatae No. 2002, Bohemia, near Pacov, 1910, representing the bunt so destructive on rye in 1910, as reported by Bubak (6); (E) From Balkosan, Turkey, coll. by G. Gassner, August 8, 1953, comm. by I. E. Niemann; (F) From Rauris, Austria, coll. Fr. Pichler, December 23, 1955. (G-I) Dwarf bunt, *T. contraversa*: (G) From Switzerland, Canton Graubunden, 1953, coll. H. Aebi, det. and comm. I. E. Niemann ("Halme Verzwergt"); (H) From Turkey (Konya-Ermenek), coll. and comm. M. Ozkan, 1954; (I) From Flora, Oregon, U.S.A., coll. and det. J. P. Meiners, August 26, 1955. All illustrations somewhat retouched to compensate for lack of sharp focus and to give perspective. × approx. 760.
Photos by Lois Johnsgard.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with little or usually no gelatinoid sheath; and (4) little or usually no dwarfing of the host.

As seen in median view the tips of the winged reticulations on the exospore of *Tilletia secalis* appear truncate as compared with the more spiny appearance of *T. contraversa.* The teliospores are mostly 21-24μ in diameter, somewhat larger on the average than in *T. caries* and *T. contraversa* if the sheath on the latter is not included. The sterile cells of *T. secalis* tend to be larger (15-24μ) and thicker walled than is true of either *T. caries* or *T. contraversa,* and in many collections they show a definite brownish tint.

There is no question that both *Tilletia contraversa* and *T. secalis* have caused severe epiphytotics of bunt in rye in parts of Europe and Asia. If the true *T. secalis* causes little or no dwarfing, as I think to be the case, then we have an explanation of why some workers have reported severe dwarfing caused by rye bunt and others have either not mentioned dwarfing or have stated that rye bunt does not cause it. Those who have observed severe dwarfing were dealing with dwarf bunt of cereals and grasses, *T. contraversa,* whereas the others were concerned with the ordinary rye bunt, *T. secalis.* Possibly some investigators have been dealing with admixtures of the two species. It seems possible also that at least some of the discrepant reports on other physiological phenomena in *T. secalis* are due to the fact that more than one species has been involved.

It will remain for my colleagues in areas of Europe and Asia where rye bunt occurs to determine the actual validity of my separation of the rye bunts. I am convinced in my own mind that (1) there are three different reticulate-spored species of *Tilletia* on rye, (2) of these, *T. secalis* has not yet been found in North America although it is widespread in Europe and parts of Asia. My studies of the comparative morphology of *T. secalis* and *T. contraversa,* based mostly on herbarium specimens, need field substantiation. Besides dwarf bunt, *T. contraversa,* on rye, there is another, perhaps more widely distributed species, that does not cause dwarfing and which has elongated, fragile bunt balls with dusty spore mass, and whose spores lack a sheath. This, I think, is the true *T. secalis.*

Summary

On the basis of a study of some 60 collections of bunt, *Tilletia* spp., on rye, as well as on published records, it is concluded that there are four distinct species of *Tilletia* on rye.

One of these species is *Tilletia foetida,* with smooth spores. It has been infrequently encountered in Argentina, Bulgaria, the North Caucasus, and the United States.

The other three species are reticulate-spored. Of these, one is the common wheat bunt, *Tilletia caries,* with comparatively shallow areolae in the exospore. The other two are dwarf bunt, *T. contraversa,* and rye bunt, *T. secalis.*

Dwarf bunt produces marked stunting in rye, has greenish black, rounded, more or less indurate spore balls, and the prominently reticulate teliospores are encased in a gelatinoid sheath. The ordinary rye bunt, considered to be *T. secalis,* does not cause dwarfing, and has bunt balls that are brownish, elongate and fragile, with a more or less dusty spore mass. The deeply reticulate exospore has little or usually no gelatinoid sheath.

The fact that there have been so many conflicting statements about dwarfing of the host and concerning various morphological and physiological characteristics of *"Tilletia secalis"* is believed to be due to the fact that two different species have been concerned: *T. contraversa* and *T. secalis.*

In the absence of type material of Corda's *"Uredo secalis",* a lectotype of *Tilletia secalis* is suggested. This is Rabenhorst's *Uredo secales,* issued as No. 1399 of Klotzsch's Herbarum Vivum Mycologicum, and which was the basis of Körnicke's binomial, *T. secalis.* Assuming, as Körnicke apparently did, that Corda's *Uredo secalis* and Rabenhorst's *Uredo secales* are the same, then the common rye bunt should bear the binomial *Tilletia secalis* (Corda) Körn. Körnicke was the first to recognize the rye bunt as a *Tilletia.*

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STUDIES ON THE PHYSIOLOGY AND NUTRITION OF *TILLETIA CARIES* AND *T. CONTRAVERSA**

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Introduction

Bunt, or stinking smut, continues to limit seriously the production of wheat in the Pacific Northwest. This problem has been aggravated by the increasing prevalence and severity of dwarf bunt (*Tilletia contraversa* Kühn.) (1,12,14), which also has recently assumed economic proportions in certain forage grasses (4,8). The causal fungus of this disease was first recorded as a variety of *T. caries* from Montana in 1935 (25). Since then it has generally been considered as a physiologic race of *T. caries* until its recent elevation to specific rank (3,4). This species differs from *T. caries* in numerous ways, but primarily in chlamydospore morphology, physiology of chlamydospore germination, disease cycle, and host symptoms (11,12,17,24). In view of the increasing prevalence of dwarf bunt in the Pacific Northwest and elsewhere, a comparative study of *T. contraversa* and *T. caries* was undertaken to determine their biological relationships. The results of certain physiological and nutritional investigations of these two species *in vitro* are reported here.

Materials and Methods

Physiologic race 9 of *Tilletia caries,* which germinates readily under controlled conditions, and a collection of *T. contraversa,* both on wheat, were used in this study. Chlamydospores of *T. caries* were germinated on 2 per cent water agar at 15° C. and those of *T. contraversa* on 3 per cent water agar at 5° C., both in 10 cm. pyrex Petri dishes. The chlamydospores were dusted onto the surface of the medium with a clean camel's hair brush. Basidiospores (secondary sporidia) of both species were germinated on 1 per cent water agar in 6 cm. Petri dishes at 15° C. The basidiospores were

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allowed to fall onto the surface of the medium by the double plate method (10), which eliminates the possible carrying over of germinated or nonviable basidiospores as well as any possible contaminations of nutrients from stock cultures. In nutrition experiments, the agar was purified with aqueous pyridine and alcohol as described by Robbins and Ma (19).

In the growth studies, Zscheile's (26) MT-2 medium was modified slightly and used as the basic medium. In this modified medium, asparagine and sucrose were used at 2.36 gm. and 10.0 gm. per liter, respectively. All chemicals were of C.P. grade, and throughout all the experiments the water used was redistilled twice through glass.

All cultures were of monosporidial origin and were grown in 125 ml. pyrex Erlenmeyer flasks containing 10 ml. of liquid medium. Stock cultures were maintained in the same medium. Quadruplicate inoculations were made by transferring a pin-head-sized colony from the stock cultures to the surface of the medium. Care was taken to float the inoculum on the surface, since neither species will grow satisfactorily when submerged. The flasks containing *T. caries* were incubated at 15° C. for 14 days, and those containing *T. contraversa* at 20° C. for 18 days. These are optimal conditions for growth, as revealed by earlier trials. In most cases growth of the cultures was measured in terms of dry weight. Before being weighed, the cultures were steamed, then washed and the mycelium recovered by filtration through filter paper, using a Buchner funnel under vacuum. The mycelium and filter papers were then dried overnight in a hot-air oven at 90° C. and weighed.

Hydrogen-ion concentrations were measured with a Beckman pH meter. Before sterilization the media were adjusted with either N/10 HCl or N/10 NaOH so that the final pH would be approximately 6.5 after autoclaving. Unless otherwise stated, the media were sterilized by autoclaving at 15 lb. pressure for 15 minutes.

Glassware was cleaned with potassium dichromate-sulfuric acid cleaning mixture, and thoroughly rinsed with tap and distilled water.

Experimental Results

I. Factors influencing chlamydospore germination.

Temperature. The temperature range for chlamydospore germination of *Tilletia caries* was found to be from 8° to 25° C. and of *T. contraversa* from 5° to 8° C. In the latter species, a minimum incubation period of 3 weeks was required. Pre-soaking of spores

(5) occasionally hastened germination, but this treatment was not essential. No germination was observed when spores of *T. contraversa* were incubated at or above 10° C. for various periods.

Nutrient requirements. Chlamydospores of *Tilletia caries* and *T. contraversa* germinated on pyridine purified water agar (2 per cent), indicating that no exogenous nutrients or growth factors were required to initiate spore germination. The addition of thiamin to water agar at 1 p.p.m. did not enhance germination in either species.

Aeration and moisture. Atypical spore germination, expressed as excessively long promycelia as described for *Tilletia cerebrina* (22), was frequently observed. There was circumstantial evidence that this was caused by excess water on the surface of the medium and deficient oxygen supply. To test this possibility, spores of *T. caries* were germinated on water agar containing 0.5, 1.0, 1.5, and 2.0 per cent agar in Petri dishes. On 0.5 per cent agar, production of both primary sporidia and basidiospores was suppressed in over 65 per cent of the germinating spores. On 1.0 per cent agar 24 per cent of the spores germinated atypically, while on 1.5 and 2.0 per cent agar only 5 per cent of the spores germinated abnormally.

In an experiment to determine the effect of aeration, spores were incubated on 2 per cent water agar in Petri dishes. Rubber seals were applied to some of the Petri dishes to limit the oxygen supply, while others were left unsealed. More than 75 per cent of the spores in the sealed dishes germinated atypically in contrast to only 5 per cent in the control plates. It is thus apparent that any condition which affects the supply of oxygen or the amount of moisture on the surface of the medium may markedly modify the type of germination.

In vitro *fungitoxicity of actidione.* Contradictory results have been reported as to the extent of practical control of stinking smut of wheat (*Tilletia caries*) with actidione (cycloheximide) in the field and greenhouse (9,13). In attempting to learn the possible cause of these discrepancies, an experiment was set up to determine the *in vitro* fungitoxicity of actidione to chlamydospores of *Tilletia caries.* Germination tests were made on 2 per cent water agar, which was previously adjusted to pH 5, containing actidione concentrations ranging from 0.6 to 1.8 p.p.m. The results are summarized in Table I.

TABLE I. PERCENTAGES OF CHLAMYDOSPORE GERMINATION OF TILLETIA CARIES AFTER 7 DAYS' INCUBATION AT 15° C. ON 2 PER CENT WATER AGAR CONTAINING VARIOUS AMOUNTS OF ACTIDIONE

Actidione conc. in p.p.m.	0.0	0.6	0.8	1.0	1.2	1.4	1.6	1.8
Percentage germination	98.2	19.6	14.0	11.7	5.4	4.3	2.9	0.0

It is obvious that actidione has a marked toxic effect against *T. caries* in culture, completely inhibiting chlamydospore germination at 1.8 p.p.m. All germination which occurred on media containing more than 1.0 p.p.m. actidione was atypical, and the production of basidiospores was greatly arrested.

II. Factors influencing basidiospore germination.

Experiments were made to determine the effects of temperature, medium reaction (pH), nutritional requirements, and the presence of actidione and indole-3-acetic acid on the germination of basidiospores of *Tilletia caries* and *T. contraversa.*

Temperature. The temperature range for basidiospore germination of *Tilletia caries* and *T. contraversa* was found to lie between 2° and 28° C., the optimum being 15° to 20° C. Within this optimum temperature range, basidiospores germinated in less than an hour, sending out germ tubes either from one or both ends. On nutrient agar these germ tubes grew rapidly into hyphae which produced crops of basidiospores in 24 hours. Rate of the germination process was slower at the lower temperatures. At 5° C. or below, visible signs of germination were observed only after 12 hours.

Nutrient requirements. More than 95 per cent of the basidiospore germination of *Tilletia caries* and *T contraversa* was obtained on pyridine purified water agar (2 per cent), indicating that no exogenous nutrients or growth factors were required to initiate germination. The addition of 1 mg. glucose and 2.5 mg. $MgSO_4.7H_2O$ per 10 ml. medium greatly enhanced germination and also mycelial development.

In studying the physiology of basidiospore germination of a number of haplonts of *Tilletia caries,* Halbsguth (7) obtained no more than 50 per cent germination on several complex media. He suggested that these media, particularly potato-peptone-agar, contained substances that inhibited basidiospore germination. To test this possibility, 9 combinations of natural media used by Halbsguth

(7) were prepared according to his directions. In repeated trials more than 95 per cent germination of basidiospores of *T. caries* was obtained on each medium.

The influence of sucrose concentration on basidiospore germination was also investigated. In both species the basidiospores germinated freely on agar media containing sucrose in amounts ranging from 0.125 up to 16.00 per cent. The optimum sucrose concentration appeared to be between 4.0 and 8.0 per cent.

The presence of certain chemical substances.

Actidione. In earlier experiments, it was found that pure actidione checked chlamydospore germination at very low concentrations. Similar tests were made on basidiospore germination of *Tilletia contraversa.* The results, presented in Table II, show that

TABLE II. PERCENTAGES OF BASIDIOSPORE GERMINATION OF TILLETIA CONTRAVERSA ON 0.5 PER CENT AGAR AT 5 ACTIDIONE CONCENTRATIONS AT 15° C. AFTER 24 HOURS

Actidione Concentration p.p.m.	Plus Glucose (1 mg. per ml.)	Minus Glucose
0.0	100.0	100.0
0.1	100.0	100.0
0.2	97.0	73.5
0.4	67.0	47.5
0.8	9.0	14.0
1.6	0.0	0.0

basidiospore germination was inhibited at increasing concentrations of actidione, with complete inhibition at 1.6 p.p.m. With one exception there were slightly higher percentages of germination at the corresponding concentrations of actidione if a small amount of glucose (1 mg. per ml.) was added (Table II).

Indole-3-acetic acid. Indole-3-acetic acid has been reported to inhibit growth of *Tilletia caries* at concentration of 4×10^{-10} (2). It is not clear whether this reduced growth was primarily due to a failure of basidiospore germination or to subsequent development following germination. Germination tests were made on 0.8 per cent sucrose agar containing indole-3-acetic acid 0.1, 1, 5, 10, 50, and 100 p.p.m. Germination was inhibited entirely at 100 p.p.m., and only 50 per cent germination occurred at 50 p.p.m. At lower levels germination was not reduced.

III. Factors affecting growth in culture.

Temperature. Monosporidial lines of *Tilletia caries* and *T. contraversa* were grown in the basic medium at 6 different temperatures ranging from 5° to 30° C. Results (Table III) show that

TABLE III. EFFECT OF TEMPERATURE ON GROWTH OF T. CARIES AND T. CONTRAVERSA IN CULTURE

Species	Temperature 5	10	15	20	25	30°C.
	Dry Weight in Mg.					
T. caries	+	6.8	27.5	24.3	5.5	0
T. contraversa	+	5.4	25.5	31.3	4.0	0

+=Slight growth 0=No growth

although both fungi can grow in a range of from 5° to 25° C., abundant growth occurred only at 15° to 20° C. Outside the optimum range growth continued longer at the lower temperatures than at the higher ones. For example, at 5° C. there was continuous growth when the cultures were incubated more than 3 weeks. However, no further growth was noted at 25° C. after 3 weeks.

pH of the medium. In investigating the influence of pH of the medium on the growth of *Tilletia caries* and *T. contraversa,* a complete medium minus phosphates was prepared, and various amounts of H_3PO_4, KH_2PO_4, K_2HPO_4, and K_3PO_4 were used as buffers, at 8 levels covering a pH range from 3.7 to 9.2. The total phosphate ion concentration was kept constant at 0.02 M. The phosphate buffers as well as thiamin chloride were sterilized by filtering through a sintered glass filter and then added aseptically to the nutrient solutions. The pH of each mixture was checked with a Beckman pH meter, and the buffer concentrations were found to be satisfactory.

It was found that the pH range for growth of both fungi extended from a minimum of 3.7 to a maximum of 9.2, the optimum being from 6.1 to 7.6. *T. caries* appeared to grow slightly better on the alkaline side than did *T. contraversa.*

Carbon nutrition. Recent investigations by Halbsguth (7) and Zscheile (26) have given information on the carbon and nitrogen nutrition of *Tilletia caries. T. contraversa* has not been investigated previously in this respect. A comparative study of the utilization of a number of carbohydrates, alcohols, and amino acids as sources of carbon for both species was therefore undertaken. The sugars

and alcohols were first tested at 0.5 per cent in the basal medium minus sucrose. The asparagine in the original medium was replaced by 2.86 gm. of ammonium nitrate. The results as summarized in Table IV indicate that the requirements for the growth of both species were identical.

TABLE IV. COMPARATIVE EFFECTS OF VARIOUS CARBON SOURCES ON GROWTH OF TILLETIA CARIES AND T. CONTRAVERSA IN CULTURE

Carbon Source	Amount of Growth of *T. caries*	Amount of Growth of *T. contraversa*
No sugar added	0	0
Monosaccharides		
L-(+) Arabinose	0	0
D-(+) Dextrose	++++	++++
D-(+) Galactose	++	++
D-(−) Levulose	++++	++++
D-(+) Mannose	±	±
L-(+) Rhamnose	0	0
L-(−) Sorbose	0	0
D-(+) Xylose	0	0
Disaccharides		
D-(+) Cellobiose	+	+
D-(+) Lactose	±	±
D-(+) Maltose	++	++
Saccharose	++++	++++
Trisaccharides		
D-(+) Raffinose	+++	+++
Polysaccharides		
Dextrin	++	++
Insulin	++	++
Soluble starch	0	±
Alcohols		
Dulcitol	0	0
i-Erythritol	0	0
Mannitol	±	+
D-Sorbitol	±	±
Glycoside		
Salicin	+	+

++++ Excellent growth
+++ Good growth
++ Moderate growth
+ Slight growth
± Trace
0 No growth

In another experiment, 6 sugars (dextrose, levulose, maltose, mannose, raffinose, and sucrose) were further tried at a concentration of 1.0 per cent. In both species it was found that dextrose,

levulose, and sucrose gave best growth, followed by raffinose and maltose. Mannose did not appear to support any growth at all.

An attempt was also made to determine if any amino acids can be utilized by *Tilletia caries* and *T. contraversa* as carbon sources. Nine amino acids were selected and tested at an amount of 420 mg. carbon per 100 ml. medium. The amino acids were added individually to the basic medium either with or without sucrose at 500 mg. per 100 ml. The amino acids tested were: DL-alanine, arginine·HCl, asparatic acid, glycine, glutamic acid, L-histidine·HOl, DL-isoleucine, L-leucine, and L-valine. Each medium was adjusted to pH 6.5 before sterilization. None of the amino acids was found to support or increase the growth of *T. caries* or *T. contraversa,* either in the presence or absence of sucrose.

Sucrose concentration. The influence of sucrose concentration on the growth of *Tilletia caries* and *T. contraversa* was investigated by growing cultures on the basic medium with sucrose concentrations ranging from 0 to 240 gm. per liter. The results are presented diagrammatically in Figure 1.

It is apparent from these results that the optimum sugar concentration for growth of *Tilletia contraversa* was 2 per cent, while that of *T. caries* was 4 per cent. When the sugar concentration was increased, the production of basidiospores of both species was proportionally retarded. On media of high sugar concentration the cultures appeared as compact, raised mycelial colonies composed mainly of pseudoparenchymatous hyphae. This was in contrast to the formation of many loose, small, scattered colonies bearing numerous basidiospores at the lower sugar content. This heaped type of mycelial growth on solid media with high sugar concentration had been previously noted by Kienholz and Heald (15) and by Flor (6), but no mention was made of its relationship to the formation of basidiospores.

Nitrogen nutrition. The availability of various nitrogen compounds in the growth of *Tilletia caries* and *T. contraversa* was investigated by growing each species in the basic medium, modified to contain different nitrogen sources. In the first experiment, 11 organic nitrogen compounds were used. The amount of nitrogen used in each treatment was equivalent to 50 mg. nitrogen per 100 ml. The pH of the medium was adjusted to 6.5 before autoclaving. Both sodium nitrite and urea were sterilized by filtration. The results, as presented in Table V, show that both fungi can utilize

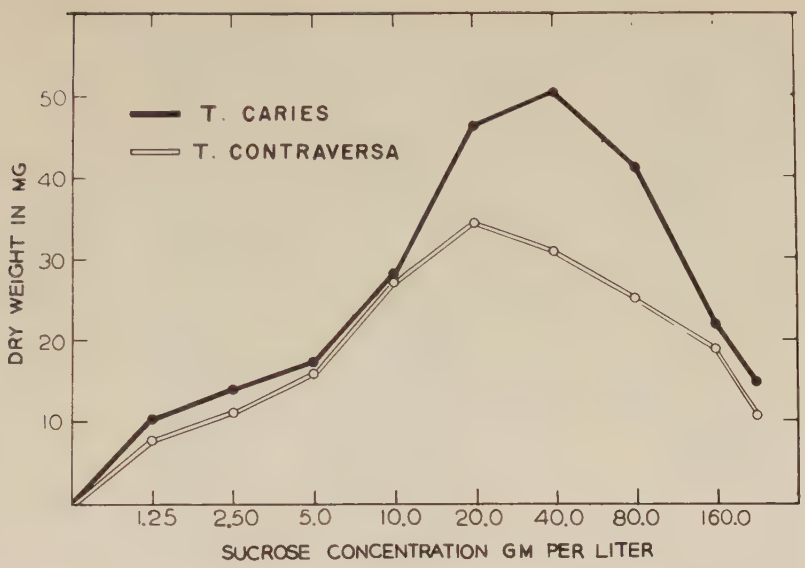

Figure 1. Influence of sucrose concentration on growth of *Tilletia caries* and *T. contraversa* in culture.

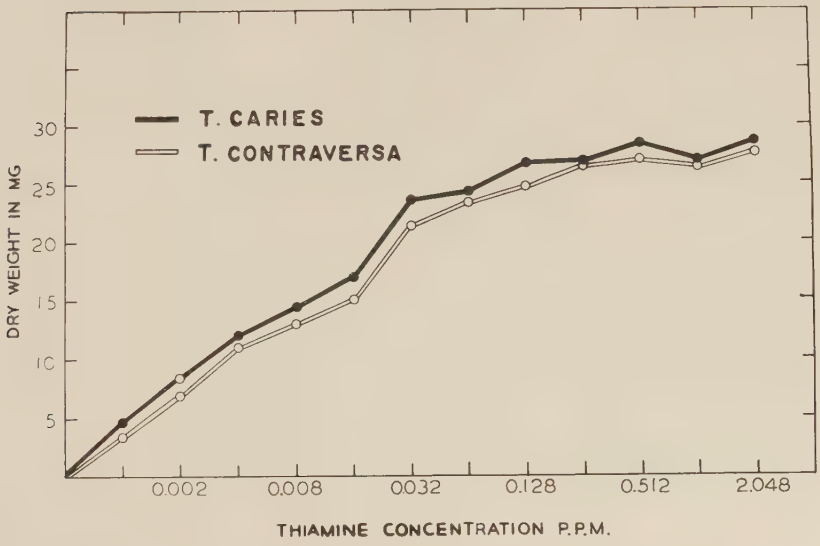

Figure 2. Growth responses of *Tilletia caries* and *T. contraversa* to various concentrations of thiamin.

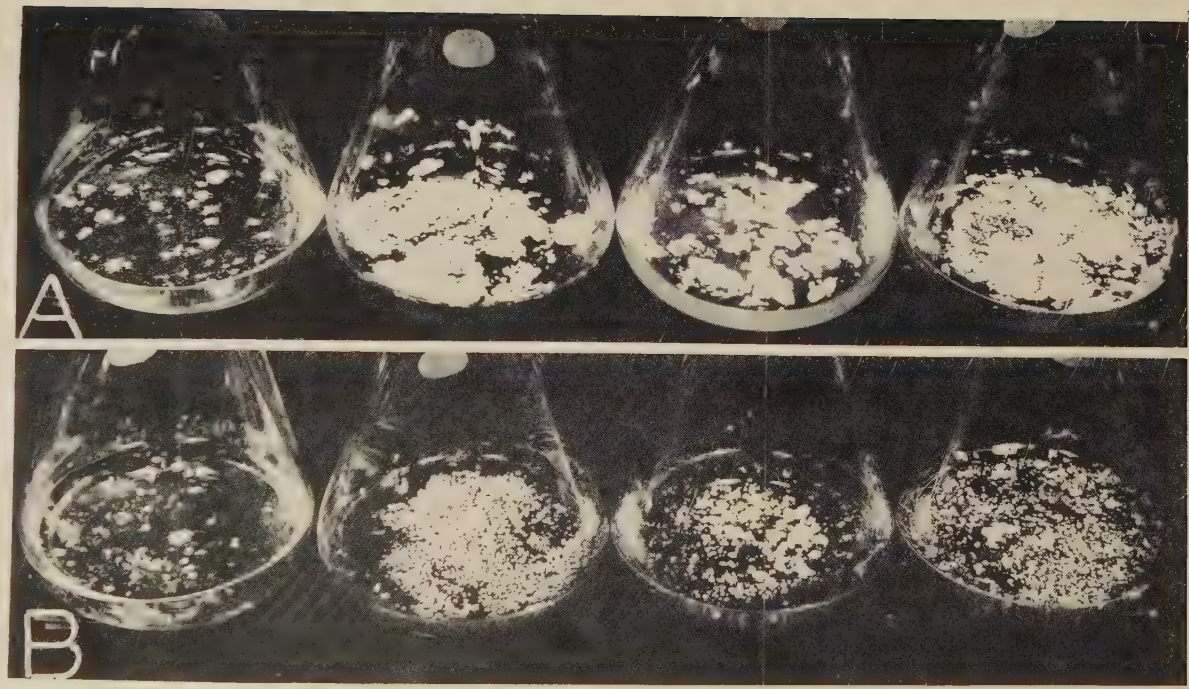

Figure 3. Growth of *Tilletia caries* (above) and *T. contraversa* in pyrimidine, thiazole, and thiamin. From left to right: pyrimidine, 6.0 p.p.m.; thiazole, 4.0 p.p.m.; pyrimidine, 6.0 p.p.m. plus thiazole, 4.0 p.p.m.; and thiamin, 10.0 p.p.m.

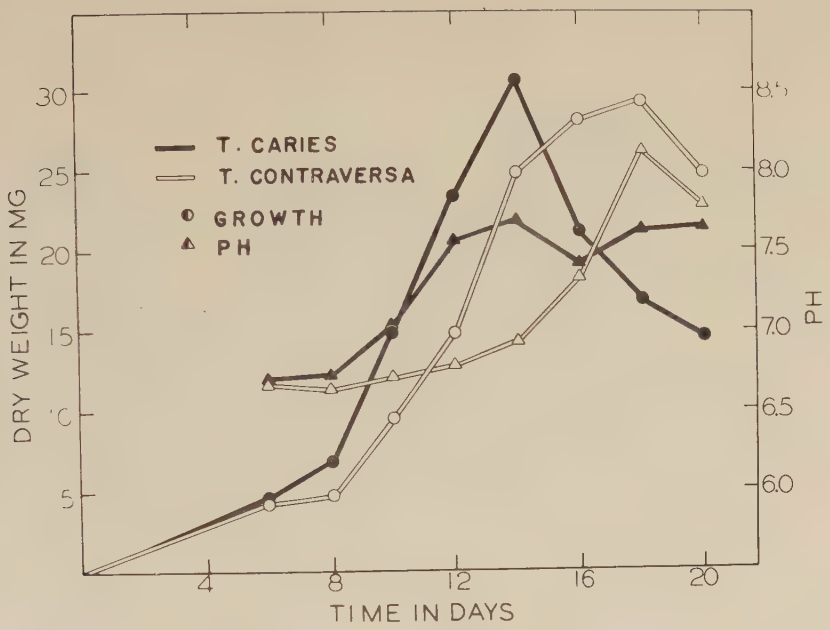

Figure 4. Growth-curves of *Tilletia caries* and *T. contraversa* in culture.

TABLE V. COMPARATIVE EFFECT OF VARIOUS NITROGEN SOURCES ON GROWTH OF TILLETIA CARIES AND T. CONTRAVERSA IN CULTURE

Nitrogen Source	Initial pH	Final pH *T. caries*	Final pH *T. contraversa*	Dry Wt. in Mg. *T. caries*	Dry Wt. in Mg. *T. contraversa*
No nitrogen	6.7	6.7	6.6	0.0	0.0
NH_4NO_3	6.2	3.2	3.5	6.5	5.0
$(NH_4)_2SO_4$	6.4	3.4	3.6	5.3	7.5
$(NH_6)_2C_4H_4O_6$	6.2	4.9	5.3	6.0	4.5
NH_4Cl	6.2	3.0	3.3	5.8	4.0
KNO_3	6.8	7.3	7.3	12.8	7.8
$NaNO_3$	6.7	7.6	7.4	13.8	7.5
$NaNO_2$	6.5	7.1	7.3	9.3	10.0
Urea	6.6	8.0	8.2	18.5	17.8
Asparagine	6.7	7.0	6.8	29.8	26.3
Casein hydrolysate "vitamin free"	6.7	7.2	6.1	31.0	23.5
Peptone	7.0	7.3	6.9	25.8	26.0

many kinds of nitrogen compounds, including ammonium, nitrate, nitrite and organic forms. The organic forms are much more readily utilized than inorganic ones. When both NH_4 and NO_3 ions are present, the former are believed to be utilized first, as shown by the development of acidity when NH_4NO_3 was supplied. It is probable that the slight growth of both species in ammonium compounds might be largely due to the drop in pH of the medium.

In another experiment, an attempt was made to learn if any amino acid will serve as a good nitrogen source for both species. Fourteen amino acids were added singly to the basic medium as a sole nitrogen source at a concentration of 50 mg. per 100 ml. The results are presented in Table VI.

It is evident that among the 14 amino acids tested, all except DL-methionine and DL-threonine may serve as nitrogen sources for both species, although most of them can only be slightly utilized. Good growth was attained only in arginine·HCl, asparatic acid, glycine and glutamic acid.

Thiamin and its moieties in relation to growth. In investigating the growth responses of *Tilletia caries* and *T. contraversa* to thiamin and its moieties, two monosporidial lines were grown in the basic medium, as in earlier studies. Two ingredients of the medium, asparagine and sucrose, were purified with Norit A. Thirteen different levels of thiamin, ranging from 0.001 to 4.096 p.p.m. in

TABLE VI. COMPARATIVE AVAILABILITY OF 14 AMINO ACIDS IN THE GROWTH OF TILLETIA CARIES AND T. CONTRAVERSA AS NITROGEN SOURCES

Nitrogen Source	Growth *T. caries*	Growth *T. contraversa*
No nitrogen	0	0
Asparagine	++++	++++
DL-Alanine	±	+
Arginine·HCl	+++	+++
Asparatic acid	+++	+++
Glycine	++	+++
Glutamic acid	+++	+++
L-Histidine·HCl	+	++
DL-Isoleucine	+	++
L-Leucine	++	++
DL-Lysine·HCl	±	+
DL-Methionine	0	0
DL-Phenylalanine	+	+
DL-Threonine	±	0
DL-Tryptophane	+	+
DL-Valine	+	++

++++ Excellent growth
+++ Good growth
++ Moderate growth
+ Slight growth
± Trace
0 No growth

a geometrical series, were tested. Results obtained from both species show that they are heterotrophic for thiamin. There was very feeble growth when thiamin was not added to the medium. Growth responses were shown at a concentration of 0.001 p.p.m., becoming more evident at 0.032 p.p.m. No significant additional effect was observed when the concentration was above this level (Fig. 2).

In further experiments, the growth responses of these two species to two thiamin intermediates (2-methyl-5-ethoxymethyl-6-amino pyrimidine and 4-methyl-5B-hydroxyethyl thiazole) were investigated. Thiazole was applied at concentrations of 0.04, 0.4, and 4.0 p.p.m., and pyrimidine at 0.06, 0.6, and 6.0 p.p.m. These two compounds, at the same concentrations, were applied either singly or together. When supplied together, the sum concentration of each constituent at 3 levels tested would be equal to a concentration of 0.1, 1.0, and 10.0 p.p.m. of thiamin, respectively. The results are presented in Table VII (Fig. 3).

TABLE VII. COMPARATIVE GROWTH RESPONSES OF TILLETIA CARIES AND T. CONTRAVERSA TO THIAMIN AND ITS MOIETIES IN CULTURE

Substrate Conc. p.p.m.	Dry Weights, in Mg. *T. caries*	*T. contraversa*
Thiazole		
0.04	13.0	12.8
0.40	20.5	16.5
4.00	20.0	18.2
Pyrimidine		
0.06	5.0	4.6
0.60	5.3	5.2
6.00	5.8	6.8
Thiazole-Pyrimidine		
0.04 + 0.06	18.0	18.2
0.40 + 0.60	22.0	20.0
4.00 + 6.00	24.5	22.4
Thiamin		
0.10	22.5	21.0
1.00	24.2	23.5
10.00	26.8	25.0

It was apparent that there was no significant difference in amount of growth in either species whether thiamin or its moieties were supplied. Apparently, both species are able to synthesize the thiamin molecule from its two components, and an exogenous supply of intact thiamin molecule is not necessary. Much better growth was obtained when thiazole was added than when pyrimidine was supplied, indicating that both species can synthesize pyrimidine but probably not thiazole. In addition it was also noted that slight acidity was developed in cultures when pyrimidine alone was added.

The presence of certain chemical substances. The effect of indole-3-acetic acid, alpha-naphthalene acid, maleic hydrazide, and actidione on the growth of *Tilletia caries* and *T. contraversa* was investigated. An amount of each chemical ranging from 1×10^{-10} to 1,000 p.p.m. was added to the medium aseptically after having been sterilized by filtration. The amount of growth of *T. caries* and *T. contraversa* was reduced approximately 50 per cent in the presence of 100 p.p.m. indole acetic acid. Neither reduction nor increase of growth was noted when the concentration used extended from 1×10^{-10} to 1 p.p.m. No effect on growth was exerted by alpha-naphthalene acid or maleic hydrazide when these two

substances were added from 10 to 100 p.p.m. Growth was completely checked when actidione was added at 5 p.p.m. or more.

Time-growth curve. The results of experiments on carbon and nitrogen nutrition of *Tilletia caries* and *T. contraversa* revealed that growth was at a maximum when sucrose and asparagine were supplied as carbon and nitrogen sources, respectively. The growth rates of both species at their respective optimum temperatures were determined by measuring their dry weights and pH changes of the nutrient solutions every other day through a period of 20 days. Maximum dry weights of *T. caries* were obtained after 14 days, and *T. contraversa* after 18 days, as shown in Figure 4. There were comparable changes in the pH values of the nutrient solutions during the growing period.

Discussion

There is a significant difference in the temperature requirements for chlamydospore germination of *Tilletia caries* and *T. contraversa.* Confirming the reports of earlier workers (11, 12, 17), *T. contraversa* germinates only through a limited temperature range, from 5° to 8° C. Treatments with various chemicals failed to induce chlamydospore germination in this species. Thus far, low temperature appears to be the only critical factor known. In this respect, *T. contraversa* resembles certain species of *Tilletia* on wild grasses (5, 22).

Nutrient media have been reported as unsuitable for chlamydospore germination in *Tilletia* (18, 23). Halbsguth (7) also found that complex potato decoction, peptone, and substances containing sugar inhibit basidiospore germination of *T. caries,* but that this effect might be mitigated by the addition of charcoal to the medium. In the present experiments, over 95 per cent basidiospore germination of this species was obtained on 9 different combinations of natural media on which Halbsguth was unable to get more than 50 per cent germination. This discrepancy might be due to different physiologic races used in the tests. It seems also likely that the age of cultures is another critical factor, since the writer has found that many basidiospores from relatively old cultures have lost their ability to germinate.

In studies by earlier investigators, artificial culturing of the wheat fungi was mainly on natural or semi-synthetic media. Particular attention was given to cultural characteristics, and little to specific nutritional requirements. Defago (2) was apparently the

first to grow *Tilletia caries* in a purely synthetic nutrient solution. The importance of using chemically defined media has been stressed recently by other workers (7, 16, 26).

In physiological or nutritional studies of *Tilletia,* one difficulty encountered is how to determine the amount of growth. Linear measurement on solid media is inapplicable because mycelial growth in these fungi is very limited. Furthermore, the addition of agar may possibly introduce certain unknown growth substances. In liquid culture, most haplonts do not grow very well when submerged, even with constant shaking. It might be for this reason that Halbsguth (7) did not use liquid media in his studies despite many difficulties in determining the dry weights of the colonies from solid media. However, he attributed the fluctuations in dry weights in his experiments to the source and size of the inocula, type of growth, and other unknown factors. It would seem that liquids are preferable, since their chemical composition can be well defined. In this investigation, it was observed that many haplonts of *T. caries* and *T. contraversa* would grow satisfactorily in liquid media if the colonies were kept from being submerged. The determination of the dry weights of liquid cultures eliminates many difficulties usually encountered in such a determination with cultures from solid media, particularly when the amount of growth is scant.

The carbon and nitrogen requirements of *Tilletia caries* and *T. contraversa* were found in this study to be essentially alike. Sucrose, dextrose, and levulose were the three most satisfactory sugars for both species. These two species differed, however, in their optimum sugar concentration requirements. For *T. caries,* the optimum sucrose concentration was 4 per cent, while for *T. contraversa* it was 2 per cent. Zscheile (26) reported that levulose was a poor substitute for sucrose. In the present investigation, levulose was found to be as good as sucrose or dextrose. Maltose was found to be a poor source of carbon, and lactose was utilized by neither species. In a report by Sartoris (20), maltose was listed as better than levulose, and lactose was listed as being utilized. Since this investigator apparently grew his cultures in sole sugar solutions, his data cannot be compared with those of workers who used complete media.

It has been confirmed that organic forms of nitrogen were more readily utilized than others (7, 16, 26). Growth was very

poor when inorganic nitrogen compounds were supplied. Nitrates were better than ammonium compounds. The poor growth of both species in ammonium compounds was believed to be due to the development of acidity in cultures. "Vitamin free" casein hydrolysate and peptone gave very good growth, but the composition of these two compounds is not definite. Among a number of amino acids and amides tested, L-asparagine gave the best growth. DL-alpha-Alanine was a very poor source of nitrogen, which confirms Zscheile's (26) findings and is in contrast to Halbsguth's (7). This discrepancy might be due to the presence of different amounts of D- or L- forms in the chemical used, as Zscheile (26) demonstrated that L-asparagine gave 10 times as much growth of *T. caries* as the D- form.

The growth responses of *Tilletia caries* and *T. contraversa* to thiamin were noticeable at a concentration of 0.001 p.p.m., the optimum being 0.032 p.p.m. These data are in general agreement with the findings of Defago (2), Halbsguth (7), and Zscheile (26) but in contrast to those of Schopfer and Blumer (21) for *T. caries.* The optimum concentration at 0.032 p.p.m. was in complete agreement with Halbsguth's figure of 0.040 p.p.m. for *T. caries,* although both Defago and Zscheile had reported a lower optimal concentration. The requirement of thiamin can be met by administration of the thiamin moieties, pyrimidine and thiazole at proper concentrations. Both *T. contraversa* and *T. caries* are thiazole heterotrophic but can synthesize their own pyrimidine. The report by Halbsguth (7) that *T. caries* was pyrimidine heterotrophic might be due to the use of old cultures, as he discussed in his paper. It appeared that, besides variations due to different races used, the differences in thiamin requirements may also be affected by the amount of the sugar supplied, length of incubation period, temperature of incubation, and possibly many others.

Summary

The effects of certain external factors on the germination of chlamydospores and basidiospores of *Tilletia caries* and *T. contraversa* have been investigated. It was demonstrated that neither species required exogenous nutrients or growth factors to initiate germination.

The influence of temperature and of acidity of the medium on the growth of *Tilletia caries* and *T. contraversa* was studied. The

optimal temperature was found to be 15° and 20° C. for *T. caries* and *T. contraversa,* respectively. Both species were able to grow through a pH range of 3.7 to 9.2, although *T. contraversa* did not grow so well on the alkaline side as did *T. caries.*

Among 21 compounds tested, sucrose, dextrose, and levulose were found to be the most satisfactory carbon sources for both *Tilletia caries* and *T. contraversa.* Raffinose was a fairly good source. Gelactose, maltose, dextrin, and insulin were all poor carbon sources, and lactose was not utilized at all. The optimal sucrose concentration for *T. caries* was 4 per cent; for *T. contraversa,* it was 2 per cent.

Among 9 amino acids tested, none could be utilized as a carbon source by either species.

Concerning nitrogen nutrition, organic compounds, such as peptone and casein hydrolysate, were much more readily utilized than inorganic forms. L-asparagine appeared to be the only amide known to induce growth comparable to that in peptone and casein hydrolysate.

Both species were heterotrophic for thiamin. These requirements can be met by supplying the two components of thiamin (pyrimidine and thiazole) at proper concentrations. Both species can synthesize pyrimidine but require thiazole for good growth.

In culture, *Tilletia caries* grows faster than *T. contraversa.* Under their most favorable growth conditions, maximum dry weights of *T. caries* were obtained after 14 days, and of *T. contraversa* after 18 days.

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GERMINATION RESPONSES OF *USTILAGO TRITICI* (PERS.) ROSTR. TELIOSPORES IN RELATION TO LYOPHILIZATION

I. SOME FACTORS AFFECTING MORTALITY BEFORE AND AFTER SUBLIMATION*

S. O. Graham†

Introduction

Maintaining the viability of teliospores of certain species of *Ustilago* beyond a few months is one of the limiting factors in studies on their biology, pathogenicity and genetics. Usually stocks of viable spores of such species are maintained by periodic passage through the host plant, a procedure that risks mutation and/or contamination as complicating factors in genetic and pathogenicity studies. Application of the lyophile process may prove helpful in prolonging viability of teliospores of short-lived smut species as it has been shown to increase spore longevity of other fungi (6, 11, 12).

In practice, the lyophile process employs this principle: if pressure over a solution is reduced below 4 mm. Hg. (the vapor pressure of ice at 0° C.), drying will occur from the frozen state. A sublimation of the ice occurs; i.e., water is removed as vapor without passing through a liquid phase. Hence, the ice scaffolding of a frozen, salt-proteinaceous material (such as the protoplast of a spore) disappears, leaving the protein and salt molecules separate in a dry molecular skeleton resembling a spongy mass with a volume equal to that of the original frozen material. Thus a proteinaceous material can be dried without the molecules aggregating or coming into contact with high salt concentrations, either of which would denature the proteins.

Preliminary to storage studies, it was found necessary to determine certain factors affecting mortality before and after sublimation, since variations in spore germination were noted, depending upon procedures and suspending media. This paper deals with teliospore collections[1] of *Ustilago tritici* (Pers.) Rostr, having high initial

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[1] Collections made on June 6, 1955 from field infections were obtained from Dr. Wayne Bever, University of Illinois.

germination levels as their viability was affected by various treatments before and after lyophilization.

Materials and Methods

Apparatus

A lyophile apparatus patterned after that of Raper and Alexander (10) was employed. This apparatus consisted of a Machlett-type, pyrex glass, freeze-dry unit which functioned as a condenser, and a manifold attachment designed to allow sublimation of thirty samples simultaneously. The vacuum source was a Cenco "Hy-Vac" pump with a capacity of .0003 mm. mercury. As added protection of the pump during operation, a trap immersed in the refrigerant held in a DeWar flask was fixed between the condenser unit and the pump. The refrigerant used in both the condenser and trap unit was CO_2 ice in 95 per cent ethyl alcohol.

Sample containers consisted of twenty-milliliter neutraglas ampules. Drying time was six hours, with ampules exposed to room temperature during operation.

The selection of media for suspending spores during lyophilization was based on results of various workers (2, 3, 5, 9, 12, 15, 16) who obtained high survival of bacterial cultures. Initially these media were compared with water suspensions and with dry spores in regard to their effect on spore survival during the various phases of the lyophile process.

The base mediuim was *"Mist Desiccans"*, developed by Fry and Greaves (5). It consists of three parts serum, one part nutrient broth (3 g. beef extract, 10 g. peptone per liter) with seven and one-half per cent glucose added. In this study the medium was labeled Solution A.

A modification of this base medium was made to incorporate additional factors demonstrated by Fry (4) and Stamp (15) to have bearing on longevity of lyophilized cultures as well as prevention of initial kill brought about by the lyophile process. Fry (4) has shown that egg yolk increases the life of lyophilized cultures of bacteria and aids in preventing initial killing from quick freezing. Both of these effects were attributed to its high colloidal content. Stamp (15) has shown that addition of ascorbic acid to the suspending medium aids in reducing initial kill of bacteria brought about by the lyophile process, and assists in prolonging the life of lyophilized cultures by its antioxidant effect.

Based on these reports a modified medium was made consisting of two parts Solution A and one part egg yolk. Ascorbic acid was added to make a concentration of .25 per cent. This suspending medium was labeled Solution B.

Spore germination measurements

To standardize spore germination measurements, conditions were selected which have been shown to be optimum for culture (8). The culture medium used in all tests was potato-sucrose agar.[2] Incubation temperature was 22° C. Standardized procedures in measuring germination consisted of seeding three plates of the agar with spore suspensions for any given treatment. One hundred spores were counted from three different locations on each plate. The mean percentage germination for the nine separate counts was used as the level of germinability for any given treatment at any given time. Percentage germination was recorded hourly for eight hours or longer, as the case demanded.

Experiments

To gain a better insight on the lethal effects of lyophilization on teliospores, the various phases involved in the process were investigated. Procedures were followed as outlined by Raper and Alexander (10) to pin-point where and when loss of germinability occurred. In addition, imposing certain conditions which might foster better germination of teliospores was considered as a possible factor in post-treatment of lyophilized cultures. Toward these objectives, the following series of experiments was conducted.

The effect of quick freezing on spore germination

Two-milligram samples of teliospores were quick frozen in the dry state, in two-milliliter suspensions of distilled water, and in two-milliliter suspensions of Solutions A and B. The shell-freezing procedure of Flosdorf and Mudd (2) was employed in quick freez-

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[2] Potato-sucrose agar formula consists of: (1 Liter)

Potato (infusion from)	400 g.
Sucrose	60 g.
Malt Extract	20 g.
Peptone	1 g.
Agar	20 g.

For convenience, a Difco, dehydrated product was used for this medium. However, this company calls the above formulation "Bacto Potato Malt Agar, Dehydrated."

ing, using a temperature of —38° C. Freezing was initiated as soon as possible after placing spores in suspension. Table I shows

TABLE I. GERMINATION RESPONSES OF USTILAGO TRITICI TELIOSPORES QUICK FROZEN IN DIFFERENT STATES AND SUSPENDING MEDIA

Treatment	Spore State or Suspending Medium	Per Cent Germination in Hours							
		1	2	3	4	5	6	7	8
Check	Dry	0	0	Trace	21.4	50.7	69.2	96.0	97.3
	Water	0	0	Trace	16.9	49.7	72.7	94.7	97.7
	Solution A	0	0	Trace	11.4	25.4	56.1	77.3	91.3
	Solution B	0	0	0	Trace	20.7	65.6	91.2	95.8
Quick Frozen	Dry	0	0	0	4.4	17.8	42.2	73.6	90.4
	Water	0	0	0	1.4	3.2	5.7	5.7	5.6
	Solution A	0	0	0	0	6.9	10.0	25.7	46.0
	Solution B	0	0	0	0	3.2	8.7	33.7	56.0

the effect of quick freezing on the germinability of *U. tritici* teliospores under the conditions outlined above. It is obvious from these data that dry spores were less affected by quick freezing than spores quick-frozen in suspensions. Quick freezing in a water suspension caused greater mortality than quick freezing in Solutions A and B. Mortality was somewhat less in Solution B than in Solution A. The reason for this was not determined.

The difficulties experienced in making hourly counts of germinated spores from Solutions A and B are apparent from the inconsistent germination levels of the check series shown in the table. Unless the promycelium was well defined, so as to limit confusion due to ergastics in the medium, the spore was not counted as germinated at any particular reading. Therefore, some spores which had really germinated undoubtedly were not counted because the promycelium had not elongated enough to be well defined. In any event, the colloidal suspending media give some protection against the killing effect of quick freezing. Although such a factor is immaterial to the life cycle of loose smuts, it fosters the idea that soil colloids may have considerable bearing on viability of overwintering spores in the soil.

There was a noticeable lag of initial spore germination after quick freezing treatments. The reason for this is not known. It was consistent in all tests involving freezing. It is interesting to note, however, that in dry spore samples there was compensation for this lag during latter portions of the test period. Because of

high mortality, comparable germination response rates of spores from suspensions were indeterminable, although the indications were present. Results were clouded by a false lag in solutions A and B, caused by difficulties in distinguishing the short promycelia of just-germinated spores which were masked by ergastics in these media.

The effect of lyophilization without previous quick freezing on spore germination

Two-milligram samples of teliospores in the dry state, and in two-milliliter suspensions of water, Solution A and Solution B, were lyophilized without previous quick freezing. Ampules containing suspensions were agitated after the degassing phase, but during auto-freezing, to prevent breakage. After sublimation had been completed, vacuum was broken and two milliliters of distilled water was administered to each ampule to rehydrate and suspend spores. Suspensions were plated-out and germination counts made according to standard procedures described above. Results are presented in Table II.

TABLE II. Germination Responses of Ustilago tritici Teliospores after Lyophilization Without Preliminary Quick Freezing

Spore State or Suspending Medium	Per Cent Germination in Hours							
	1	2	3	4	5	6	7	8
Dry	0	0	0	2.3	23.3	56.3	82.3	95.8
Water	0	0	0	1.4	8.8	18.0	31.3	41.7
Solution A	0	0	0	Trace	19.2	32.5	49.2	56.5
Solution B	0	0	0	6.0	24.2	37.5	51.7	74.0

Again it is noted that dry spores were less affected by the treatment than similar samples lyophilized from suspensions. The same relative positions were maintained in regard to suspending media, although there was considerably less kill of spores suspended in water. If the principal loss of viability of spores is directly related to the manner of freezing, then these data support the results of Haskins (6), who has shown that slow freezing brings about less killing of certain fungus spores than quick freezing. Killing, then, brought about by lyophilization *per se,* may be merely a portion of the kill due to freezing. In order to distinguish any difference between losses brought about by the quick freezing process and freezing *per se* in the lyophile process, one must combine the two factors and compare.

The lag in percentage germination of lyophilized spores, if plotted against time as compared with untreated checks (Table I), is essentially the same as the lag noted in the quick freezing tests. It would appear that perhaps additional counts might have shown additive levels of germination of treated spores. However, additional counts were made up to sixteen hours, and very little if any change in percentage germination occurred. This point was checked several times, because it seemed likely that some difference would manifest itself. It can only be speculated that in addition to the lag in germination due to treatment there is an earlier peak in germination of treated spores from suspensions as well.

The effect of quick freezing followed by lyophilization on spore germination

Two-milligram samples of teliospores in the dry state, in two-milliliter suspensions of distilled water, and in two-milliliter suspensions of Solutions A and B, were quick-frozen in the manner described earlier and then lyophilized. To prevent any possible thawing of the sample before the auto-freezing vacuum level was reached, ampules holding the quick-frozen samples were removed from the refrigerant and attached immediately to the lyophilizer, which was in operation at its maximum capacity.

On completion of sublimation, vacuum was broken and two milliliters of distilled water were administered to each vial for rehydration and suspension of spores. Suspensions were plated-out and germination counts made according to standard procedures already described. Counts are shown in Table III. It is apparent

TABLE III. Germination Responses of Ustilago tritici Teliospores Quick Frozen and Then Lyophilized

Spore State or Suspending Medium	Per Cent Germination in Hours							
	1	2	3	4	5	6	7	8
Dry	0	0	Trace	6.9	29.1	61.1	79.7	93.3
Water	0	0	0	0	0	Trace	1.7	4.5
Solution A	0	0	0	0	3.6	9.9	16.1	18.6
Solution B	0	0	0	0	1.9	8.4	15.0	26.0

from these data, as it was in previous tests, that germinability of spores in the dry state is affected very little by either quick freezing or lyophilization. In fact there was no greater loss of viability in dry spore samples where quick freezing was followed by lyophili-

zation than there was from similar samples which were quick-frozen without being lyophilized.

However, when the data from Table III are compared with data from Tables I and II, it is apparent that spores placed in suspensions did not follow this same pattern. In analyzing the pattern produced, relative to suspensions, it appears that loss of germinability occurred at least twice. The losses were additive in these cases, contrary to the losses as they occurred when dry spores were treated in a similar manner. One loss occurred during the quick freezing treatment and at least one other during the process of lyophilization or during the rehydration of spores in plating-out.

In order to determine as nearly as possible what role rehydration might have in the loss of germinability of lyophilized teliospores, post-treatment of spores was investigated from the standpoint of rate of moisture uptake.

Post-lyophilization humidity treatment of spores

Conditioning of lyophilized cultures of teliospores before plating-out may have considerable effect on germinability, since it is known that autolysis occurs in rehydration of animal tissue after freeze-drying (1). Rowell (11) has shown that quick uptake of water by rust uredospores which had been lyophilized brought about mechanical separation of the spore wall from the protoplast by differential rehydration. He considered that this differential rehydration might be the lethal factor in reconstitution of lyophilized rust spores.

On the thesis that initial uptake of moisture by sublimed teliospores in a water-saturated atmosphere would be slow, lyophilized teliospores were placed under such an atmosphere for 24 hours prior to plating-out. Germination responses from this treatment were compared with those not receiving such post-lyophile conditioning (Table IV).

Dehydrated solutions A and B, being very hygroscopic, absorbed moisture in a very short time (two hours or less). In 24 hours the spores were suspended in a syrupy medium. However, there had been no germination.

Spores lyophilized from the water suspension were very hygroscopic themselves and were quite moist after 24 hours in the saturated atmosphere. They had not germinated either.

In contrast, spores lyophilized from the dry state did not absorb

moisture to a great degree and were found to be as difficult to suspend as untreated check spores. This would indicate an alteration in the surface tension of spores lyophilized from suspensions. Such an alteration induced by aqueous suspensions may be of considerable importance in defining lethal effect of quick freezing and/or lyophilization.

Comparing results shown in Table IV with those from Tables I, II, and III, it is apparent that there were two well-defined

TABLE IV. GERMINATION RESPONSES OF LYOPHILIZED TELIOSPORES AFTER CONDITIONING IN A WATER-SATURATED ATMOSPHERE FOR TWENTY-FOUR HOURS

Spore State or Susp. Medium	Treatment* Plus Conditioning in Hours	Per Cent Germination in Hours							
		1	2	3	4	5	6	7	8
Dry Spores	Ck+0	0	0	Trace	21.3	50.7	69.2	96.0	97.3
	Ck+24	0	Trace	10.5	33.1	60.2	82.4	86.9	96.3
	L+0	0	0	0	2.3	23.3	56.3	82.3	95.8
	L+24	0	Trace	19.6	71.0	81.0	92.7	95.8	97.7
	QF—L+0	0	0	Trace	6.9	29.1	61.6	79.7	93.3
	QF—L+24	0	Trace	27.1	78.7	93.7	95.4	97.0	98.3
Water	Ck+0	0	0	Trace	16.9	49.7	72.7	94.7	97.7
	Ck+24	0	Trace	12.1	32.8	61.2	79.3	93.2	96.6
	L+O	0	0	0	1.4	8.8	18.0	31.3	41.7
	L+24	0	Trace	18.7	30.2	44.6	50.3	56.0	57.2
	QF—L+0	0	0	0	0	0	Trace	1.7	4.5
	QF—L+24	0	0	1.9	2.1	2.1	1.8	2.3	3.3
Solution A	Ck+0	0	0	Trace	11.4	25.4	56.1	77.3	91.3
	Ck+24	0	Trace	9.3	29.6	48.8	69.9	90.2	92.1
	L+0	0	0	0	Trace	19.2	32.5	49.2	56.5
	L+24	0	Trace	12.7	27.3	46.3	58.1	64.0	69.7
	QF—L+0	0	0	0	0	3.6	9.9	16.1	18.6
	QF—L+24	0	0	Trace	6.7	18.2	19.6	24.4	28.9
Solution B	Ck+0	0	0	0	Trace	20.7	65.6	91.2	95.8
	Ck+24	0	0	Trace	16.3	39.9	58.7	89.2	94.0
	L+0	0	0	0	6.0	24.2	37.5	51.7	74.0
	L+24	0	Trace	8.7	19.4	37.0	66.1	77.1	82.8
	QF—L+0	0	0	0	0	1.9	8.4	15.0	26.0
	QF—L+24	0	0	3.3	9.6	24.5	31.1	38.2	37.9

* Ck=check; L=lyophilized; QF—L=quick frozen and then lyophilized.

phases when loss of viability occurred if spores were treated in suspensions, *viz.*, during quick freezing and during rehydration. One would be inclined to postulate from these results, a third loss during the lyophile process, for it is obvious that if quick freezing alone prejudiced the germinability of teliospores, then quick freezing followed by vacuum desiccation would be unlikely to improve the outcome. However, such additive losses in germinability were only apparent when spores were treated while in suspension. Any additional losses incurred after quick freeezing might be entirely due to the manner in which rehydration is practiced. Dry spores, quick-frozen, lyophilized and then conditioned prior to plating-out showed germinability levels comparable to untreated spores.

Factors in rehydration must not only involve prevention of mechanical separation of the protoplast from the spore wall or some other mechanical damage which might be lethal (Rowell, 11), but must also take into account the eutectics of salts in the dehydrated protoplast, wherein physiologic inactivation of enzymes might occur when slow uptake of water is involved.

Since dry spores were least affected by the process as a whole, it would appear from these data that such material should be used in anticipation of storage. However, merely because higher initial germination levels were obtained by following this procedure, there is no reason at present to assume that lyophilized dry-spore samples as a whole will have an extended storage life greater or as great as spore samples lyophilized from suspensions. The protective effects of colloids and other materials in conjunction with storage must be considered.

The evidence is conclusive, however, that quick-freezing pretreatment of teliospore samples before lyophilization is to be avoided. It serves no particular purpose, and results are contradictory to a common belief that it is necessary: to reduce mechanical injury to cell structure, quick-frozen ice crystals being much smaller than slow-frozen ones; and to pass over the eutectic points of salt solutions, thus preventing the build-up of injuriously high salt concentrations. These considerations, although possibly valid for cells containing large quantities of water, seem unimportant in the sublimation of drought-resistant teliospores of *Ustilago tritici*.

Since the mature, dry teliospore is obviously in a drought-resistant condition for dissemination, it is by nature cold-resistant as well, for physiologic drought resistance and physiologic cold resist-

ance are equivalent. In view of the resistance of dry teliospores to low temperature, coupled with post-conditioning as a means to reduce mortality on rehydration, further study will be given to determining the effect of this treatment on prolonging viability of teliospores of *Ustilago tritici* and other smut fungi which die in a short time under current storage practices.

Summary

Using a lyophile apparatus patterned after that of Raper and Alexander, the various phases of the lyophile process as it has been used in their procedures were investigated to pin-point where and when loss of germinability occurred in lyophilization of short-lived teliospores of *Ustilago tritici.*

It was found that dry teliospore samples retained near maximum germination levels after quick freezing, while samples placed in suspensions did not, although high colloidal content of the suspending medium limited loss of germinability somewhat. Egg yolk plus ascorbic acid added to such a medium reduced mortality attributable to quick freezing, but by masking promycelial initials, it interposed difficulty in determining exact germination rates.

If samples in suspensions were lyophilized without previous quick freezing, mortality was lower than when lyophilization was preceded by quick freezing. Dry spores, however, were affected no more by quick freezing followed by lyophilization than they were by quick freezing alone.

When lyophilized samples were conditioned for twenty-four hours in a water-saturated atmosphere prior to plating-out, marked increases in spore germination resulted. This indicates a loss of germinability in the reconstitution phase of lyophilized spores.

It is concluded that presublimation quick freezing in the lyophile process as used is not only unnecessary but detrimental to *Ustilago tritici* teliospores preliminary to storage. It is also concluded that a preconditioning of spores prior to plating-out is essential to the increased potential of samples removed from sublimation.

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PROBLEMS IN THE DELIMITATION OF SPECIES OF *TILLETIA* OCCURRING ON WHEAT*

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Introduction

Before 1938 only two species of *Tilletia* were generally recognized as causing bunt of wheat. These are separated by the fact that the spores of one [*Tilletia foetida* (Wallr.) Liro] are smooth and those of the other [*T. caries* (DC.) Tul.] reticulate. Early workers (1, 2, 17, 18) described the spores of *T. caries* as having winged reticulations with mesh widths ranging from 2μ to 7μ; later workers (4, 9) reported even greater differences. Furthermore, physiologic races of *T. caries* may differ in this characteristic (6, 10, 14). Although such differences have not heretofore been regarded as valid for species delimitation, the current trend is to recognize additional species of *Tilletia* on wheat by differences in the spore wall reticulations and in certain other characters as well. The purpose of this paper is to explore the implications of a multiple-species concept in this group, especially with reference to spore wall reticulations.

Literature Review

Gassner (7) was first to set up taxonomic entities of *Tilletia* on the basis of differences in spore wall markings when he established *T. caries* v. *intermedia* because its spores had exceedingly fine "reticulations." Savulescu *et al.* (20) expanded Gassner's concept in a report on the distribution of four "species" (*T. caries, T. foetida, T. triticoides* Savul., *T. intermedia* Gassner) occurring on wheat in Rumania. Hulea (16) supplemented this with an extensive dissertation on the biology of these four "species," and Grasso (8) reported the occurrence of the same four "species" in Italy. Fischer (5) described *T. brevifaciens* G. W. Fischer as a fifth species on wheat and certain grasses in the United States, a species that later was reduced to synonymy and assigned to *T. contraversa* Kühn by Conners (3).

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Gassner's (7) designation of an intermediate type as *Tilletia caries* v. *intermedia* was based on its fine "reticulations" which resembled *T. caries* and a thin membrane which resembled *T. foetida.* This type was found only where there was close association of the two main species, and Gassner suggested that it might be a hybrid. Flor (6) had previously reported intergrading types associated with both species in collections of smut from commercial wheat fields and had also suggested their possible origin as hybrids between *T. caries* and *T. foetida.* He observed all degrees of intergradations in the reticulate markings, ranging from exceedingly fine to so coarse as to appear spiny. Similar types occurring in Argentina were described by Hirschhorn (9), who likewise suggested that they might be of hybrid origin.

Apparently Savulescu *et al.* (20) had no difficulty in distinguishing between types or kinds of markings on the spores. These workers elevated Gassner's v. *intermedia* (7) to specific rank and established still another species category in the reticulate spore group, designating it as *T. triticoides.* Extensive studies on the biology of these four "species" led Hulea (16) to conclude that they are practically identical in all respects except morphology and rate of spore germination. It appears from their reports (16, 20) that no intergrading morphological types were encountered and, as illustrated by drawings, the differences in reticulations they observed are marked indeed. These differences, however, appeared much less striking in their photomicrographs (16).

In 1935 Young (22) called attention to "a new variety" of *Tilletia caries,* distinguishable by spore morphology and certain characteristic symptoms of the diseased host. This disease subsequently became recognized in widely separated areas and has been designated by some as dwarf bunt, caused by *T. caries* (19). It seems evident from Hulea's (16) description of four "species" that Savulescu *et al.* (20) were distinguishing between dwarf and common bunt in their *T. tritici* and *T. triticoides,* respectively, There is no indication, however, that they recognized their *T. tritici* as the causal agent of dwarf bunt.

Intensive study by Fischer (5) and Warmbrunn (21) led to the conclusion that dwarf bunt of winter wheat and certain grasses should be recognized as a separate disease from common bunt, and Fischer (5) named its causal agent *Tilletia brevifaciens,* a species distinct from *T. caries* in several respects. Among the differences

were those characteristics reported by others (11, 14) and a gelatinous sheath enveloping each spore, which was "sometimes missing on some spores" (5).

It is evident from this review that there is a broadening acceptance of a multiple-species concept with respect to the species of *Tilletia* on wheat. Although the validity of this concept probably is justifiable under the rules of nomenclature, its practicability is debatable, considering currently existing wide variability and overlapping tendencies in the distinguishing characteristics. Still greater and more complex variability may be expected, in view of the close genetic relationships between these species, and especially where the dwarf and common bunt types both occur in the same field.

Experimental Results

There is experimental evidence that hybrids between *Tilletia caries* and *T. foetida* give rise to types intermediate in size and in spore markings (12). This evidence has been corroborated by subsequent studies on a wide variety of hybrid spores involving several physiologic races of these two species. In the various generations of these experimentally produced hybrids there was a wide intergrading range of morphological types, extending from those resembling one parent species to those resembling the other parent. That a similar situation exists in the natural occurrence of *Tilletia* spp. on wheat is implied in several reports (6, 7, 9), and this is corroborated by observations on spore types found in bunt collections from commercial wheat fields in the Pacific Northwest.

The practical problem involved is illustrated in Figure 1, showing spores from four collections of common bunt. The surface view reveals the pattern of spore markings; the median view exhibits the spores in full diameter, showing not only spore size and the relative prominence of the surface markings, but the thickness of the enveloping "gelatinous" sheath as well. Two distinct and two intermediate types are represented. Those at A may be regarded as fairly typical of the species (*Tilletia caries*). Those at D are sufficiently distinct to qualify as a different species, characterized by the cerebriform surface markings and the relatively thick "gelatinous" sheath.

A problem of species delimitation develops, however, in connection with the intermediate types shown at B and C (Fig. 1), neither of which is typically reticulate nor typically cerebriform. Although

both tend to be cerebriform, those at B are more similar to the reticulate type shown at A, while the type at C resembles more closely the cerebriform type at D. On the other hand, when seen in median view, the enveloping sheath is more prominent in B than in C, and in this respect B is more like D while C is more like A. Thus there is variation in both the surface markings and the enveloping sheath, and the overlapping range of this variability extends from the cerebriform to the reticulate types, with no distinct point of separation. The self-evident implications of this situation are even more complex where both the dwarf and common bunts are involved.

Spore morphology, plant height, spore germination, and certain life cycle features are said to separate *T. contraversa* (dwarf bunt) from *T. caries* (common bunt) (5, 21). Both experimentally produced and natural hybrids between these two types have been reported (12, 13), and in both kinds of hybrids the segregating populations contained types intermediate with respect to all differentiating characteristics, further emphasizing the difficulty of delimiting species of *Tilletia* on wheat.

A preliminary report (13) on natural hybridization between common and dwarf bunt described types intermediate with respect to plant height, spore balls, spore morphology, and spore germination. Further examination of this material has confirmed the preliminary report on its hybrid nature. In this study 90 smutted heads from plants that were intermediate in height were classified both as to head type and spore type. The sori of dwarf bunt separate the palea and lemma and show between them (5), producing a fairly characteristic head type. On the basis of this head type, 34 of the above smutted heads were classified as dwarf bunt and 56 as common bunt.

In spore type, 47 of the 56 common bunt head types were identified as *T. caries,* 4 were classed as dwarf bunt (*T. contraversa*), and 2 appeared to be *T. intermedia.* The remaining 2 heads had cerebriform spores similar to those in Figure 1D, a type different from any yet reported in natural occurrence on wheat.

Among the 34 dwarf bunt head types, 30 had spores like dwarf bunt (*T. contraversa*), 2 had spores like common bunt (*T. caries*), and the other 2 had spores like *T. intermedia.* In some cases admixtures of smooth spores (*T. foetida*) with the intermediate type were seen in both the common and dwarf bunt head types.

Classification as to spore type in this natural hybrid population did not necessarily correspond to incubation period and temperature requirements for spore germination in common and dwarf bunt spores. Some of the spores that were identified morphologically as dwarf bunt (*T. contraversa*) germinated under conditions favorable for the germination of common bunt spores (*T. caries*). On the other hand, some of those classified morphologically as common bunt spores, germinated best under conditions favorable for germination of dwarf bunt spores. Still others germinated equally well at temperatures favorable for germination of both common and dwarf bunt spores.

Obviously, in this material there was sufficient overlapping of those characteristics that usually distinguish between *T. caries* and *T. contraversa* to confuse, if not preclude, positive identification of some members of the population as distinct species causing dwarf and common bunts. Furthermore, in some cases, the identity of *T. intermedia* likewise was uncertain, since there was much greater range in the intermediate types observed in this material than is indicated in the description of this "species" (19).

Discussion

These observations on the wide variability and overlapping tendencies in those characteristics that are used as species criteria in *Tilletia* on wheat would seem to suggest the need for a more practical species concept in this group. Such a concept should take cognizance of the basic characteristics that promote the development of intergrading and overlapping types. For example, smoothness as opposed to reticulation of spores is unquestionably a valid species criterion for separating *T. foetida* and *T. caries*. However, the life cycles of these two species are basically identical, and for this reason natural commingling probably provides ample opportunity for interspecific hybridization. Under these circumstances the development of intermediate or intergrading types is inevitable, because of the interaction of genetic factors. The resultant wide variation in types has confused the species concept for this group.

Though distinct for *Tilletia caries* and *T. foetida,* characteristic differences in spore wall markings are inadequate for separating such species as *T. caries, T. contraversa,* and *T. intermedia,* as shown by the overlapping in types of markings often encountered (Fig. 1, 2, 3). Although type specimens of these species may differ markedly, the

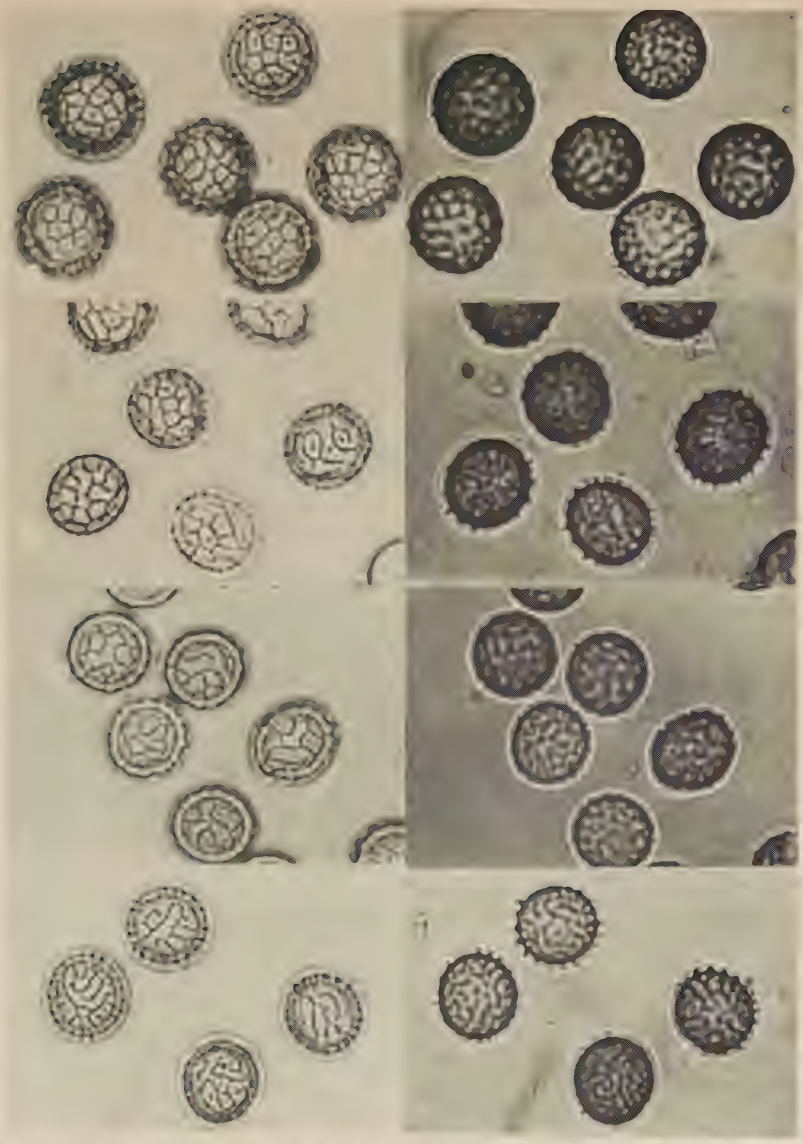

Figure 1. Photomicrographs of chlamydospores from 4 collections of common bunt, illustrating the intergrading tendencies (*B* and *C*) between distinctly different types (*A* and *D*). (Surface and median views at left and right, respectively.)

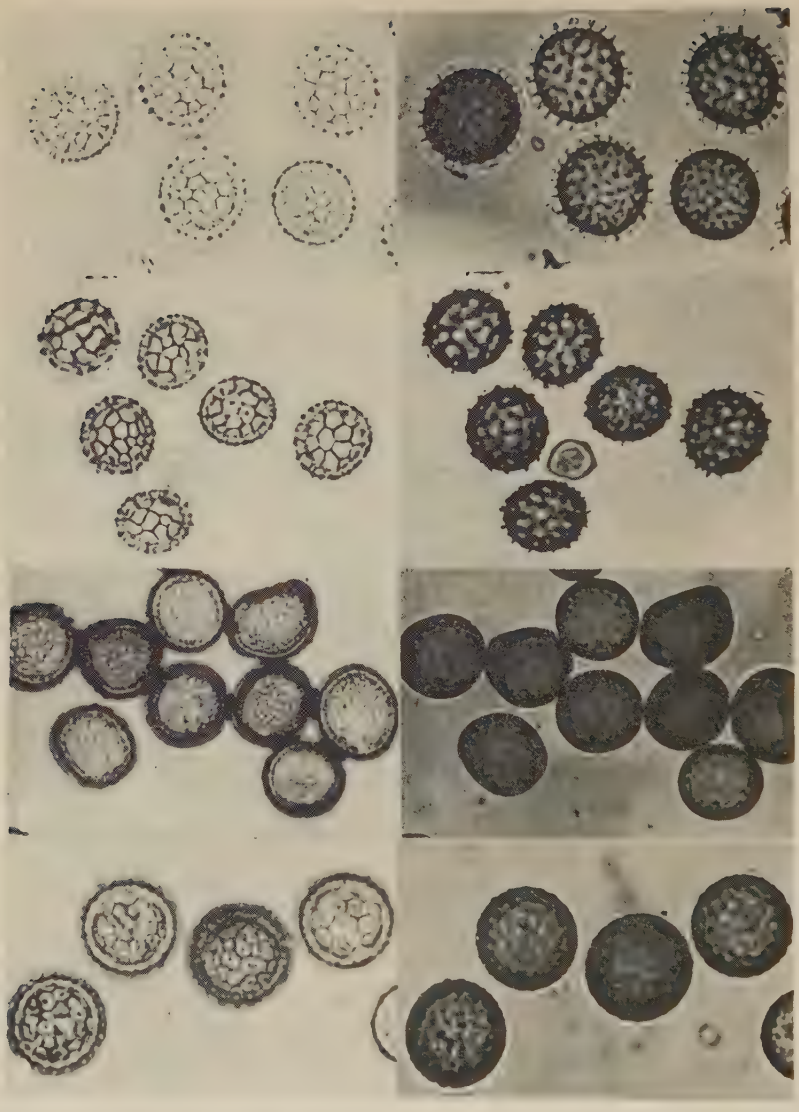

Figure 2. Photomicrographs of spores from 4 collections of dwarf bunt (*T. contraversa*), illustrating some of the variability encountered in the morphology of spores from different collections of dwarf bunt in the Pacific Northwest. Note differences in spore size, in surface markings, and in the thickness of the "gelatinous" sheath. (Surface and median views at left and right, respectively.)

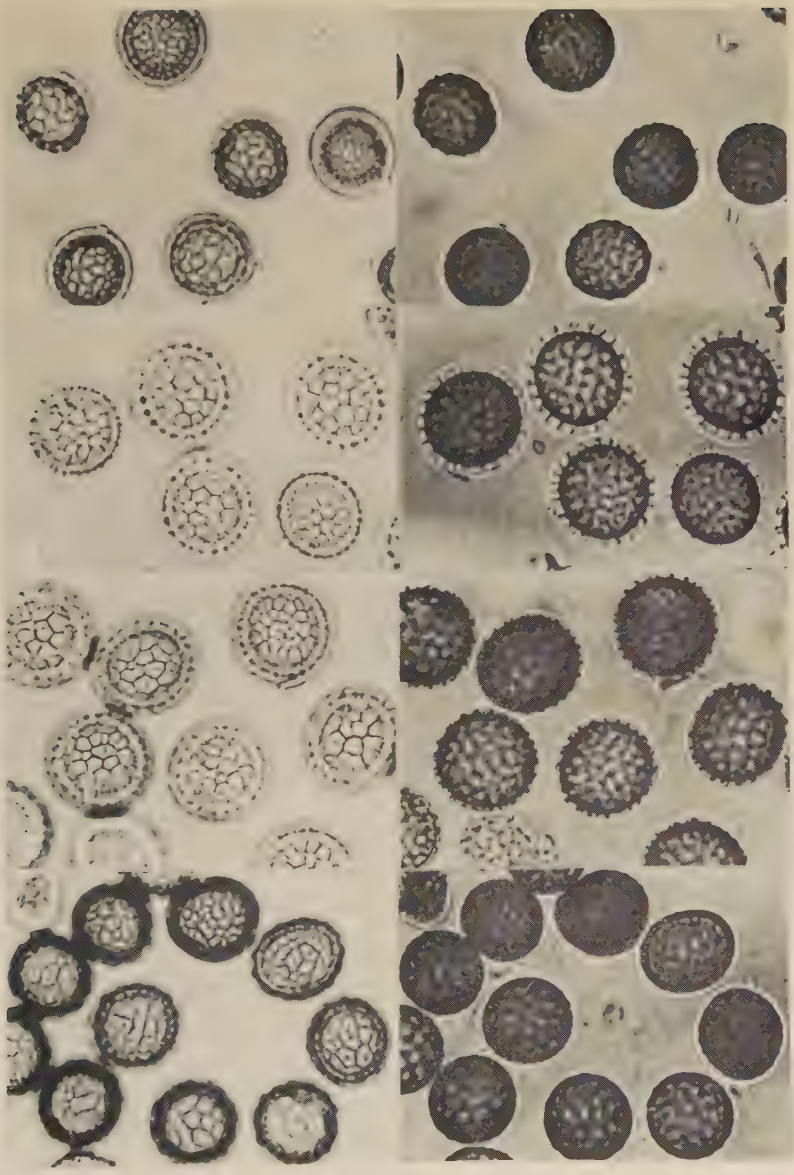

Figure 3. Photomicrographs of spores from two collections each of *Tilletia contraversa* (A and B) and *T. caries* (C and D), illustrating intraspecies differences and interspecies similarities that pose problems of identity. The spores at B and C, representing *T. contraversa* and *T. caries*, respectively, are more similar than those in A and B, representing the former species, and those in C and D, representing the latter species. (Surface and median views at left and right, respectively.)

intergrading types frequently overlap so much that no definite break between species can be established. Furthermore, certain races of *T. caries* are known to differ from each other in type of reticulation (10), and still others exhibit wide ranges in the same character within their own spore population (Fig. 3D). In other words, within the present species concept in this group, several additional species of *Tilletia* on wheat are worthy of recognition. Certainly the cerebriform type illustrated in Figure 1D is a valid species by current standards, even though its elevation to specific rank probably would create more problems of delimitation than it would solve. A case in point is *T. contraversa.*

Virtually every characteristic used in the separation of dwarf bunt (*Tilletia contraversa*) from common bunt (*T. caries*) is one of degree only. Wide ranges in type and prominence of the spore wall markings and the "gelatinous" sheath exist in both species (Fig. 1, 2), and there is inevitable overlapping between the extreme types (Fig. 3). Likewise, overlapping occurs in such characteristics as rate of spore germination, spore ball types, or dwarfing and tillering of the host. For example, excessive tillering of infected plants usually is characteristic of dwarf bunt. This was not the case, however, in two widely separated commercial wheat fields of the Pacific Northwest, where otherwise typical dwarf-bunted plants had only one tiller.

Overlapping also occurs in the degree of reduction in plant height caused by these two species. Extreme dwarfing is not characteristic of all races of *T. contraversa* (15), while some races of *T. caries* cause pronounced stunting (10). Because of these and other intergrading features it would seem impossible to establish precisely the limits of these two "species." To be sure, *T. contraversa* is distinguishable from *T. caries* on the basis of the sum total of several characteristics, as pointed out by Fischer (5) in his description of *T. brevifaciens.* If this is a valid species concept, then by the same reasoning and on the basis of seven differentiating characteristics (10), races 9 and 10 of *T. caries* might be regarded as distinct species.

Admittedly the spores of the majority of collections of dwarf bunt (*T. contraversa*) are prominently reticulate and are encased in a relatively prominent sheath. However, exceptions do occur in which spores quite unlike those described for *T. contraversa* (Fig. 2C, D) cause dwarf bunt. Furthermore, the natural compatibility

between the dwarf and common bunt types and their increasing simultaneous occurrence in the same wheat fields suggest an inevitably frequent hybridization between them. This would seem to portend the future prevalence of still other variants, many of which will qualify for specific rank by present criteria.

In dealing with known hybrid populations an accepted taxonomic practice is to refer intermediate types to the category of "hybrids." On the other hand, similar types may be recognized as new species when found in independent populations, as, for example, *T. intermedia.* Although only spore markings are involved in this case, sufficient variability in the intermediate characteristic has been encountered to establish an overlapping range of spore types between the presumed parent species. New types emanating from natural hybrids between dwarf bunt (*T. contraversa*) and the common bunt species *T. caries, T. foetida,* and *T. intermedia* are practically certain to establish independent populations.

Genetically controlled characteristics involved in hybrids between the above species may include not only differences in spore morphology but differences also in such other characteristics as spore germination, sorus type, plant height, and tiller stimulation. Hence, numerous additional variant types may be expected to be encountered with increasing frequency. Unquestionably these will further confound the problems of species delimitation in this group, unless our present concept can be revised. Perhaps broader ranges in the variability of species characteristics should be recognized; or perhaps trinomial designations should be considered.

Summary

1. Wide variability exists in some of the characteristics, especially spore wall markings, that delimit species of *Tilletia* occurring on wheat.
2. Intergradation between extreme types presents problems of identification. Natural hybridization between commingling species portends an increasing problem.
3. By currently accepted criteria at least two additional species of *Tilletia* on wheat might be recognized. One of these has spores with cerebriform reticulations and produces common bunt. The other is a dwarf bunt type with finely reticulate spores. Both types probably arose through hybridization between common and dwarf bunt.

4. Considering the potential variability inherent in this group, a revised species concept seems imperative. This might require either the use of trinomials or the acceptance of broad ranges of variability in distinguishing characteristics.

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FUSION BETWEEN PRIMARY SPORIDIA DERIVED FROM DIFFERENT SPECIES OF *TILLETIA* OCCURRING ON WHEAT AND CERTAIN GRASS HOSTS*

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Introduction

Dwarf bunt[1] of winter wheat and the species of *Tilletia* that causes it resemble in several respects certain other smuts and their respective causal agents that occur on cultivated and wild grasses. Although similar morphology and host range frequently suggest possible relationships between these smuts, more precise measures would be helpful in some cases. Fusion between primary sporidia may be of potential value, especially where genetic relationships are involved.

Fusion between paired secondary sporidia was reported recently (2) as a means of determining sex compatibility in monosporidial lines of *Tilletia caries*. It was also shown that secondary sporidia of this species will fuse with primary sporidia. Such compatibility or incompatibility between paired sporidia was suggested as a basis for indicating taxonomic relationships among species of *Tilletia* occurring on wheat and certain grass hosts. This is a report on a study of fusions between pairs of primary sporidia from some of these species.

Materials and Methods

Spores from 6 collections of dwarf bunt were used: No. 3 from Logan, Utah; No. 4 from Bozeman, Montana; No. 6 from Troy, Idaho; No. 7 from Klickitat County, Washington; No. 14 from Pullman, Washington; and No. 16 from Kalispell, Montana.

Seven species of *Tilletia* from 10 species of grass hosts were

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[1]Variously referred to as *Tilletia caries* (DC.) Tul., *T. tritici* (Bjerk.) Wint., *T. brevifaciens* G. W. Fischer, and *T. contraversa* Kühn but not clearly defined as any one of these by present criteria.

used: *T. elymi* Diet. & Holw., *T. holci* (West.) de T., *T. cerebrina* Ell. & Ev., *T. fusca* Ell. & Ev., *T. guyotiana* Har., *T. asperifolia* Ell. & Ev., and *T. caries* (DC.) Tul. The host origin of each species is shown in Table I.

TABLE I. NUMBERS OF FUSIONS THAT RESULTED FROM MATINGS BETWEEN PRIMARY SPORIDIA OF TILLETIA SPP. OCCURRING ON WHEAT AND CERTAIN GRASSES

Species Mated	Host Source	Number of Pairs of Sporidia Total	Fused
D3[a] with *T. holci*	*Holcus lanatus* L.	36	0
D3 with *T. cerebrina*	*Deschampia caespitosa* (L.) Beauv.	15	0
D3 with *T. asperifolia*	*Muhlenbergia asperifolia* (Nees & Mey.) Parodi	41	0
D3 with *T. fusca*	*Festuca pacifica* Piper	15	8
D3 with *T. guyotiana*	*Bromus japonicus* Thunb.	116	32
D3 with *T. caries*	*Agropyron trachycaulum* (Link) Malte	27	7
D3 with *T. caries*	*B. tectorum* L.	187	70
D6 with *T. caries*	*B. tectorum*	18	3
D6 with *T. guyotiana*	*B. japonicus*	28	4
D6 with *T. elymi*	*Elymus glaucus* Buckl.	30	0
D7 with *T. caries*	*B. tectorum*	39	0
D7 with *T. caries*	*B. marginatus* Nees	13	6
D4 with *T. caries*	*B. marginatus*	51	0
D4 with *T. guyotiana*	*B. japonicus*	13	1
D4 with *T. guyotiana*	*B. brizaeformis* Fisch. & Mey.	65	0
D16 with *T. guyotiana*	*B. japonicus*	31	1
D14 with *T. caries*	*B. marginatus*	39	7

[a] D denotes collections of the species of *Tilletia* that causes dwarf bunt, all of which were on wheat.

The spores of all species were germinated by incubation on 2 per cent water agar at their respective optimum temperatures, 15° C for *T. asperifolia,* 10° C for *T. guyotiana,* and 5° C for the remainder.

The pairings between primary sporidia were made on 2 per cent water agar with the aid of a Chambers micromanipulator. Complete or partial sets of the sporidia from individual germinating spores of one species were isolated and placed on the same hanging drop of medium with a comparable number of sporidia from one or more spores of another species. The number of pairs made in any one group was determined by the spore that produced the largest number of sporidia. Thus a complete set of matings with

one species might require two or more sets from another species, depending on the number of sporidia produced by individual spores of the two species. The mated sporidia were incubated usually at room temperature, but in some cases also at 10° C. In some groups where fusions occurred an attempt was made to determine the maximum number of compatible pairs. To do this the sporidia from the nonfused pairs were mated in other combinations, and this process was repeated until no further fusions occurred. This was possible, however, only if the paired sporidia from different sources were morphologically distinct.

Experimental Results

The matings show a wide range in compatibility between the sporidia of the species of *Tilletia* causing dwarf bunt on wheat and those from the species of *Tilletia* on grasses (Table I). Sporidia from dwarf bunt *Tilletia* spores on wheat fused with sporidia from *T. fusca* and *T. guyotiana,* and also with sporidia of *T. caries* from *Agropyron trachycaulum, Bromus tectorum,* and *B. marginatus.* All other species combinations gave negative reactions, as might have been expected in matings with *T. elymi* and *T. cerebrina,* both of which appear to be homothallic (1,3).

There was some evidence of difference in degree of compatibility among the compatible combinations. This seemed to be related in some instances to the dwarf bunt collection used and in others to the host from which the other species was collected (Table I). For example, combinations with collection D3 produced the greatest numbers of fusions in proportion to matings, whereas those combinations with D4 produced the least. Especially notable is the lack of fusions in combinations between D7 and *T. caries* from *B. tectorum* and in all but one of the combinations between D4 and *T. guyotiana.* Both of these were highly compatible with D3 (Table I). On a percentage basis, the combination of D7 with *T. caries* from *B. marginatus* exhibited the highest compatibility index. The total number of 13 matings in this combination, however, probably was too small to lend significance to this relatively high compatibility index. A somewhat larger number of matings between D14 and *T. caries* from *B. marginatus* produced a much smaller proportion of fusions.

The apparent difference in degree of compatibility may have been due to differential response to environmental factors, such as temperature and medium. Most of the sporidial pairs were exposed to

room temperatures, but some were incubated at 10° C, at which temperature the majority of the fusions recorded in Table I occurred. In regard to the medium, all of the matings were made on water agar, on the assumption that there would be no advantage to a nutrient medium. It has been shown (2), however, that fusion between secondary sporidia of *T. caries* occurs more readily on nutrient medium than on water agar, and this may be true with primary sporidia. Likewise, length of the incubation period may be important. For the most part the paired sporidia were incubated less than eight hours while some were held longer, up to 36 hours. The great majority of the fusions occurred between pairs that were incubated for the longest time, suggesting that more pairs might have fused if all pairs had been incubated 36 hours.

Where compatibility is exhibited in a group of paired sporidia, the nonfused sporidia may be capable of fusion in different combinations. Thus, in one group of 19 pairs (representing incomplete sets of primary sporidia from D3 and *T. caries* from *B. tectorum*), 6 pairs fused. The nonfused pairs were separated and mated in different combinations, and of these, 7 pairs fused. In an incomplete set of 16 pairs, 5 pairs fused, and the nonfused pairs were mated in other combinations, 4 of which fused. By the same procedure, 8 fused pairs were observed in another group of 17 pairs.

The above procedure, as a tool, is facilitated by a morphological distinction between the primary sporidia of the 2 species. In the above case, the sporidia of D3 were slender and flexible in contrast to those of *T. caries* from *B. tectorum,* which were stouter and more rigid. Where no morphological distinction exists the sporidia may be identified by position on the agar surface in relation to an established "landmark."

Differences in degree of compatibility between species were suggested by the development of the fused pairs of sporidia. In some combinations the fused pairs were exceedingly fragile, as shown by the fact that a slight touch with the needle point caused the structures to rupture and discharge their protoplasmic contents. If left undisturbed, however, these same fused pairs produced dikaryotic hyphae. In contrast, the fused sporidia of D3 and *T. fusca* developed no further than to complete the fusion process, whereas those of D3 and *T. caries* from *A. trachycaulum* developed small mycelial colonies. No attempt was made to follow the further development of these colonies.

Summary

Both positive and negative compatibility relationships were exhibited when primary sporidia from spores of *Tilletia* spp. occurring on wheat and on certain grass hosts were cross-mated in culture. Sporidia from spores of the species that causes dwarf bunt of wheat fused readily with sporidia from *T. guyotiana* and *T. fusca,* and also with sporidia of *T. caries* from *Bromus tectorum* and *B. marginatus,* but did not mate with *T. holci, T. cerebrina, T. asperifolia,* and *T. elymi.*

There was some evidence of different degrees of compatibility, as indicated by the percentage of mated pairs that fused and by the further development of the fused pairs.

Fusion between pairs of primary sporidia of different species of *Tilletia* has potential value as a tool in the study of taxonomic relationships.

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EXTENSION OF THE KNOWN GRASS HOST RANGE FOR *TILLETIA CARIES* BY INOCULATION*

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INTRODUCTION

Up to 1936, the known host range of *Tilletia caries* (DC.) Tul. included only rye (*Secale cereale* L.) and *Aegilops ventricosa* Tausch (1) outside of the genus *Triticum*. Inoculations reported of three other genera in the *Hordeae* tribe by Fischer (1,2) extended the host range to include 6 species of *Agropyron* [*A. cristatum* (L.) Beauv.; *A. inerme* (Scribn. & Smith) Rydb.; *A. spicatum* (Pursh) Scribn. & Smith; *A. subsecundum* (Link) Hitchc.; *A. trichophorum* (Link) Richt.; and *A. trachycaulum* (Link) Malte.] and one species each of *Hordeum* (*H. brachyantherum* Nevski) and *Sitanion* (*S. jubatum* J. G. Smith). A broader and more complex host range for common bunt of wheat (*T. caries*) is now indicated by recent reports of the natural occurrence of similar bunt diseases on grasses in the tribe *Festuceae* (4). Likewise, dwarf bunt types similar to those occurring on wheat have been reported on numerous grasses, mostly in commercial seed fields (3, 5, 6, 7, 10). These new hosts include species not only in the closely related genus *Agropyron*, but also in such unrelated genera as *Arrhenatherum, Dactylis, Festuca,* and *Bromus*.

This broadening of the host range of *T. caries* to include a wide range of wild and cultivated grasses raises the question of the relationship between bunts on grasses and those on wheat. It suggests that grasses may provide reservoirs of inoculum of wheat bunt and serve as breeding areas for the development of new races of bunt. These potentialities are being explored. The inoculation of grasses with several races of common bunt was undertaken to determine the limits of susceptibility among the more commonly occurring grasses of the Pacific Northwest.

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Materials and Methods

Nine of the standard races (11) of common bunt (*Tilletia caries*) were used in this study: T-1, T-8, T-10, T-11, T-12, T-13, T-14, T-15, and T-16. Three of these (T-8, T-11, T-16) and a hybrid selection (Hy. 119) were included as dwarf bunt tester races (8). Each of these was used to inoculate 124 accessions of grasses representing 5 tribes, 15 genera, and 72 species.[1]

The grasses were inoculated by immersing the seed in an aqueous suspension of smut spores and applying a partial vacuum. For seeds without hulls, the spores were applied as a suspension in a 5 percent methyl-cellulose sticker. After inoculation the seed was dried and planted in the nursery in April 1954. Smut readings were taken in September 1954 for those that headed the first year, and in August 1955 for those that headed the second year after planting.

Experimental Results

New hosts

Infection occurred on 30 species of grasses. Among these were 22 in the tribe *Hordeae,* 6 in the tribe *Festuceae* and 2 in the tribe *Aveneae.* Apparently this is the first time that grasses outside the tribe *Hordeae* have been shown to be hosts of wheat bunt after artificial inoculation. A brief report of these results has been published (9).

Of the 30 species of grasses that were smutted, 23 represent new hosts for *T. caries.* These are listed below, according to tribe:

Aveneae: *Arrhenatherum elatius* (L.) Presl. and *Koeleria cristata* (L.) Pers.

Festuceae: *Bromus erectus* Huds., *B. marginatus* Nees, and *B. tomentellus* Boiss., *Dactylis glomerata* L., *Festuca idahoensis* Elmer and *F. ovina* L.

Hordeae: *Agropyron amurense* Drob., *A. ciliare* (Trin.) Franch., *A. desertorum* (Fisch.) Schult., *A. dasystachyum* (Hook.) Scribn., *A. intermedium* (Host) Beauv., *A. riparium* Scribn. & Smith, *A. sibiricum* (Willd.) Beauv., *Elymus canadensis* L., *E. glaucus* Buckl., *E. giganteus* Vahl, *E. junceus* Fisch., *E. sibiricus* L., *Hordeum jubatum* var. *caespitosum* (Scribn.) Hitchc., *Lolium multiflorum* Lam., and *L. perenne* L.

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[1] Grateful acknowledgement is made to the Soil Conservation Service Nurseries, U.S.D.A., Pullman, Washington, for providing seed of most of the grasses used in this study.

No bunt was obtained on the following species:

Agrostideae: *Agrostis alba* L., *A. tenuis* Sibth., *Phleum phleoides* (L.) Karst., *P. pratense* L.

Aveneae: *Alopecurus arundinaceus* Poir. and *A. pratensis* L.

Festuceae: *Bromus catharticus* Vahl., *B. commutatus* Schrad., *B. danthoniae* Trin., *B. inermis* Leyss, *B. japonicus* Thunb., *B. macrostachys* L., *B. madritensis* L., *B. mollis* L., *B. polyanthus* Scribn., *B. rigidus* Roth., *B. rubens* L., *B. secalinus* L., *B. tectorum* L., *B. trinii* Desv., *Festuca arundinacea* Schreb., *F. octoflora* Walt., *F. rubra* L., *F. rubra commutata* Gaud., *Poa ampla* Merr., *P. compressa* L., *P. canbyi* (Scribn.) Piper, *P. longifolia* Trin., *P. nevadensis* Vasey ex Scribn. and *P. pratensis* L.

Hordeae: *Agropyron elongatum* (Host) Beauv., *Agropyron semicostatum* (Steud.) Nees, *A. smithii* Rydb., *Elymus cinereus* Scribn. and Merr., *Hordeum bulbosum* L., *H. hystrix* Roth, *H. leporinum* Link, and *Secale montanum* Guss.

Phalarideae: *Phalaris arundinacea* L.

Symptom expression

Fischer (1) observed slight to pronounced modification in the morphology of grass inflorescences affected with common bunt. Bunted heads of *Agropyron* were shorter, more compact, and plumper than normal ones, whereas bunted heads of *Hordeum brachyantherum* were not conspicuously different from nonbunted ones.

Similar observations were made in this study (Figs. 1 to 3). There appeared to be some correlation between the host genus and the degree of modification in the host morphology. With species of *Agropyron* (Fig. 1), *Arrhenatherum* (Fig. 2F), *Lolium* (Fig. 2D & E), and *Dactylis* (Fig. 3E) the inflorescences usually are greatly modified, in the manner described for *Agropyron* by Fischer (1). Therefore these are conspicuous and readily detected. In *Dactylis glomerata,* the normally compact fascicles of healthy spikelets may become even more compact when smutted (Fig. 3E), and in the case of *Arrhenatherum elatius* (Fig. 2F) the bunted panicles are somewhat more compact than the normal ones. Changes in *Agropyron desertorum* produced by the disease include proliferation and abortion of spikelets and abortion of entire portions of the spikes (Fig. 1B).

In contrast to the above genera, the bunted inflorescences of species of *Bromus* (Fig. 3A & B), *Festuca* (Fig. 3C & D), *Hordeum* (1) and *Koeleria* (Fig. 2G) were modified only slightly or not at all. In these genera difficulty is encountered in finding smutted plants in the field. Frequently this can be accomplished only by

rubbing the spikelets between the fingers to reveal the smut sori hidden within the floral bracts. The detection of smutted plants also may be facilitated by examining the shorter culms and panicles, because the smut often has a dwarfing effect. In *B. tomentellus* and *B. erectus* the smutted panicles are straw-colored in contrast to the reddish or brownish appearance of normal spikelets.

In *Elymus* the degree of morphological change is variable, depending upon the head shape of individual species (Fig. 2A, B, & C). For example, with *E. glaucus* (Fig. 2A), which has long, narrow heads similar to those of *Agropyron* spp., the smutted heads are noticeably plumper and shorter. In other species, such as *E. canadensis* (Fig. 2B), that have large, thick and lax panicles, smutted heads often can be distinguished from normal heads only when smut balls protrude from the spikelets. With both the latter species and *E. junceus* (Fig. 2C), smutted spikes are usually somewhat shorter than nonsmutted spikes.

Discussion

Bunt diseases occurring naturally on the following grasses in the Pacific Northwest have been ascribed to *Tilletia caries,* or common bunt: *Agropyron cristatum, A. trachycaulum, Bromus rigidus, B. tectorum,* and *Festuca rubra* (4). An even wider host range for dwarf bunt of wheat is indicated by the occurrence of a similar type on 12 species of *Agropyron, Arrhenatherum elatius, Bromus marginatus, Dactylis glomerata,* and *Festuca rubra* (7). These indicated relationships are based entirely on spore morphology. More specific relationships might be indicated by pathogenicity. If the bunts occurring on these grasses are pathogenically similar to those from wheat, cross inoculations would show some degree of susceptibility in both directions. The results reported here show that many grasses are susceptible to common bunt of wheat, particularly race T-16. All of the *Agropyron* species known to be natural hosts for dwarf bunt, except one (*A. elongatum*), proved to be susceptible to one or more of the common bunt races used. The dwarf bunt tester race T-16 exhibited the widest host range, and in this respect corresponds generally with dwarf bunt on grasses. *Arrhenatherum elatius* and *Dactylis glomerata,* which are susceptible to this race, are both known dwarf bunt hosts. The other dwarf-bunt-susceptible grasses, *Festuca rubra* and *Bromus marginatus,* were not bunted by T-16, but infection by this race was obtained on other species of *Festuca,* and *B. marginatus* was susceptible to race T-10 of wheat

Figure 1. Smutted (left) and non-smutted (right) inflorescences of grasses in tribe *Hordeae*: A. *Agropyron sibiricum*; B. *A. desertorum* (3 smutted spikes); C. *A. intermedium* (2 smutted spikes); D. *A. riparium*; E. *A. ciliare*.

Figure 2. Smutted (left) and non-smutted (right) inflorescences of grasses in the tribe *Hordeae*: A. *Elymus glaucus;* B. *E. canadensis;* C. *E. junceus;* D. *Lolium multiflorum;* E. *L. perenne.* Tribe *Aveneae*: F. *Arrhenatherum elatius*; G. *Koeleria cristata.*

Figure 3. Smutted (left) and non-smutted (right) inflorescences of grasses in the tribe *Festuceae*: A. *Bromus tomentellus;* B. *B. marginatus*; C. *Festuca idahoensis*; D. *F. ovina;* E. *Dactylis glomerata* (2 smutted panicles, left).

bunt. Thus the host range of dwarf bunt on grasses was almost duplicated by that of common bunt race T-16.

Race T-16 also infected several grasses not known to be hosts of dwarf bunt. These included species of *Agropyron, Elymus, Hordeum,* and *Lolium.* These possibly may represent potential hosts for the dwarf bunt race commonly attacking grasses in the Pacific Northwest.

The large number of grasses that proved to be susceptible to common bunt races after inoculation contrasts sharply with the small number of grasses reported as natural hosts. This seems surprising, in view of the fact that some of the new grass hosts reported herein are highly susceptible. One reason for this may be that in some species the smutted inflorescences are inconspicuous, hence readily overlooked in natural stands. Possibly many species may have some type of natural barrier, either physiological or ecological, which is broken down by the inoculation procedure.

Bromus tectorum, a weedy winter annual that is the most prevalent natural grass host of a common bunt type similar to *T. caries,* was not susceptible to common bunt in this series of inoculations. *B. rigidus* and several other annual and biennial species of *Bromus* did not exhibit infection.

The fact that races of common bunt of wheat may attack a wide range of hosts in the *Gramineae* is significant to both wheat and grass production. Grasses, particularly the wild ones growing in and about wheat fields, may serve as a reservoir for inoculum of wheat bunt. Since many grasses are susceptible to several races, there is the possibility that new races might arise through hybridization on these grass hosts. Similar potentialities are inherent in the occurrence of dwarf bunt on both wheat and grasses.

Summary

Inoculation studies with 10 physiologic races of *Tilletia caries* have extended the known grass host range to 23 additional species representing 8 genera and 3 tribes. Infection occurred on 30 species, of which 22 were in the tribe *Hordeae,* 6 in the tribe *Festuceae,* and 2 in the tribe *Aveneae.* Apparently for the first time, grasses other than those of the tribe *Hordeae* have been shown to be susceptible to *T. caries* by inoculation. Nearly all of the natural grass hosts of dwarf bunt were found to be susceptible to T-16, the dwarf bunt tester race of common bunt.

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PIGMENTATION OF THE CULTURE MEDIUM AS AN INDICATOR OF SEX COMPATIBILITY IN *TILLETIA CARIES**

EDGAR L. KENDRICK†

In genetic studies involving the bunt fungi [*Tilletia caries* (DC.) Tul. and *T. foetida* (Wallr.) Liro] it is essential to determine sex compatibility relationships among large numbers of monosporidial isolates. Flor (1) made such determinations on the basis of infection results from inoculations with paired monosporidial lines, and Holton (2) showed that fusion between secondary sporidia in culture is an index of sex compatibility in these species. Recently, Kendrick and Holton (3) reported that pigmentation of the culture medium is associated with sex compatibility in *T. caries.*

A reddish-brown pigmentation of the agar medium was first observed in cultures of 5 lines from spore 287 of race T-16, while the remaining 8 lines from this spore produced no such pigment. The compatibility factors of each of these lines had been determined previously by mating secondary sporidia, and it was found that those lines producing pigment were of opposite compatibility from those producing no pigment. The monosporidial lines of spore 287 represented an incomplete set of primary sporidia, since three of the 16 original isolates did not grow. A complete set of 9 lines from spore 285 of race T-16 was then examined, but no differential pigmentation was produced on potato-dextrose-agar (PDA) slants. However, pigmentation differences developed when the cultures were transferred to potato-sucrose-agar (PSA) slants, and these lines also could be separated into the two compatibility groups on the basis of pigmentation and nonpigmentation.

A study was undertaken to elucidate this pigmentation phenomenon with respect to other isolates, not only from race T-16, but from other races, as well.

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Materials and Methods

Monosporidial lines representing four races, T-9, T-10, T-15, and T-16, were used in this experiment. The lines or isolates of each race were obtained by isolating complete sets of primary sporidia from the promycelia of germinating chlamydospores. Each sporidium was transferred to a separate tube of PDA or PSA. All cultures were incubated at 15° C.

Monosporidial lines of spores 282 and 283 (T-9) and 285 and 287 (T-16) had been isolated and their compatibility factor determined prior to the start of this experiment. Nine additional complete or near complete sets of lines from T-16, five from T-10, and seven from T-15 were isolated and transferred to PSA slants. Complete sets of lines were not obtained in every case, since some of the isolated sporidia failed to grow. After the isolates had developed sufficient colony size, duplicate transfers of each were made to fresh PSA slants. When these developed into full colony size, pigmentation differences became apparent. Some cultures showed the characteristic rust-brown color; others did not. The compatibility between these two kinds of lines was then determined by matings between secondary sporidia (2).

As a standard for comparison with other sets of monosporidial lines the five pigmenting lines of spore 287, all of like compatibility, were designated as plus. The seven nonpigmenting lines, being of opposite compatibility from the pigmenting lines, were designated as minus. Thus the assignment of either plus or minus to all subsequent monosporidial lines depended on their mating reaction to the established plus and minus lines from spore 287.

For example, when all of the pigmenting lines of spore 285 were found to be of like compatibility, one of these lines was mated with a standard plus line and a minus line of spore 287. Fusions occurred only in matings with the minus (nonpigmenting) line of spore 287. Thus the pigmenting and nonpigmenting lines of spore 285 proved to be plus and minus respectively. In this manner the plus and minus designations for all monosporidial lines were determined.

The potato-dextrose-agar was prepared by adding 30 g. of Difco PDA plus 10 g. of agar to 1000 ml. of distilled water. The potato-sucrose-agar was prepared from 200 g. of potatoes, 10 g. of sucrose, and 20 g. of agar in 1000 ml. of distilled water.

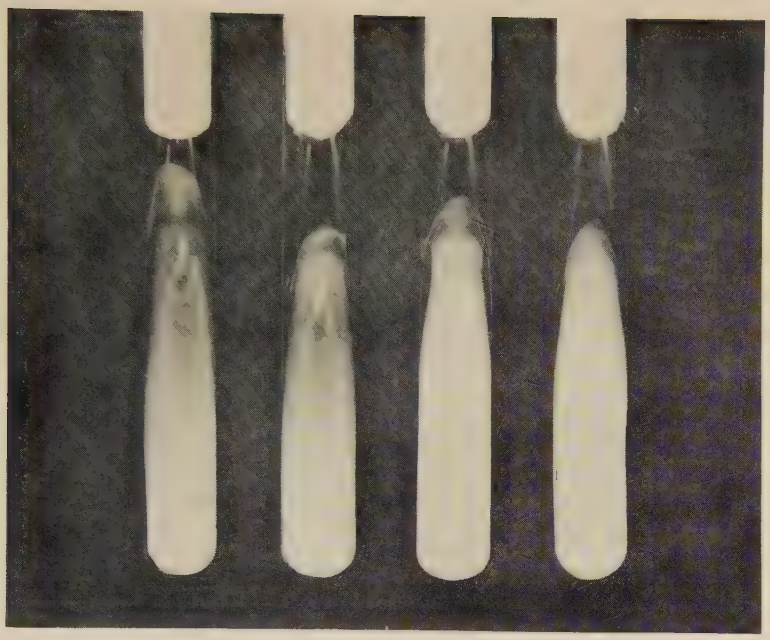

Figure 1. Two cultures each of pigmenting minus (left) and non-pigmenting plus (right) monosporidial lines from spore 350 of race T-16 of *Tilletia caries* showing the characteristic darkening of the agar medium caused by pigmentation.

Results

Effect of medium upon pigmentation

Potato-sucrose-agar, which favors a more luxuriant sporidial growth of *Tilletia caries* than potato-dextrose-agar, was found to favor the expression of pigmentation differences in the various sets of monosporidial lines. The pigmentation-compatibility relationship was first observed in lines of spore 287 on PDA. No such differential pigmentation occurred in lines of spore 285 growing on this medium. However, when the lines from both spores were transferred to PSA, the pigmentation-compatibility relationship became apparent in the lines of spore 285, and the difference between pigmenting and nonpigmenting lines of spore 287 became even more striking than it was on PDA. On PSA the pigmenting lines develop an intense reddish-brown color in contrast to the dull, light brown produced on PDA. For this reason all of the monosporidial lines were subsequently maintained on PSA.

Occurrence of the pigmentation phenomenon

Nine complete or near-complete sets of lines (spores 350-358) from race T-16, in addition to those from spores 285 and 287, were investigated with respect to pigmentation-compatibility relationship. Six out of the nine sets of lines (350, 352, 354, 355, 357, and 358) were separable into the plus and minus compatibility groups on the basis of pigmentation and nonpigmentation. Exceptions to this were the lines from spores 351, 353, and 356, which exhibited varying degrees of pigmentation that were not correlated with compatibility. For example, one set of lines (those from spore 351) could not be separated into two distinct groups of pigmenting and nonpigmenting lines. On the other hand, the lines from spores 353 and 356 could be separated into pigmenting and nonpigmenting groups, but each group contained both plus and minus lines.

Two sets of lines from spores 282 and 283 of race T-9 were likewise studied for correlation between compatibility and pigmentation. All cultures of the 11 lines from spore 282 produced a light brown color. In contrast, certain of the lines from spore 283 were classed as pigmenting while others were classed nonpigmenting. However, there was no correlation between pigmentation and compatibility in these lines. Similar results were obtained with certain sets of lines from race T-10 (302, 303, and 304). Even so, the monosporidial lines from two spores of race T-10 (300 and 309)

showed good correlation between pigmentation and compatibility.

Five out of seven sets of monosporidial lines of race T-15 exhibited the pigmentation-compatibility relationship. All the lines of the other two sets pigmented with equal intensity and therefore were not separable on the basis of pigmentation.

Relationship of pigmentation with sex compatibility

The fact that pigmentation appears to be sex-associated in certain spores from races T-16, T-10, and T-15, raised the question of whether it might also be sex-specific. Up to this point it had only been established that pigmenting and nonpigmenting lines from certain spores were of opposite compatibility. By arbitrarily designating the pigmenting lines of spore 287 as plus and the nonpigmenting lines as minus and cross-mating them with other lines, the appropriate compatibility designation was assigned to each monosporidial line in relation to these standard lines. The results of these matings for 15 sets of lines are summarized in Table I.

TABLE I. The Relation Between Sex-Compatibility and Pigmentation of the Culture Medium in 15 Sets of Monosporidial Lines Representing Three Races of Tilletia caries

Spore No.	Race	No. of Primary Sporidia Produced	Monosporidial Lines Obtained	Compatibility Groups and Pigmentation Plus (+)	Minus (—)
285	T-16	9	9	pigmenting	nonpigmenting
287	T-16	16	13	pigmenting	nonpigmenting
350	T-16	12	12	nonpigmenting	pigmenting
352	T-16	11	9	pigmenting	nonpigmenting
354	T-16	8	8	nonpigmenting	pigmenting
355	T-16	7	7	nonpigmenting	pigmenting
357	T-16	5	5	nonpigmenting	pigmenting
358	T-16	11	11	pigmenting	nonpigmenting
300	T-10	10	10	nonpigmenting	pigmenting
301	T-10	10	10	nonpigmenting	pigmenting
305	T-15	8	7	pigmenting	nonpigmenting
306	T-15	9	8	pigmenting	nonpigmenting
307	T-15	9	8	nonpigmenting	pigmenting
308	T-15	12	10	pigmenting	nonpigmenting
309	T-15	11	9	pigmenting	nonpigmenting

The data in Table I show that some of the pigmenting lines of races T-15 and T-16 were of plus compatibility and others were of minus compatibility. Thus pigmentation in these lines, although

sex-associated, is not sex-specific. In the two sets of lines from race T-10 (300, 301) all of the pigmenting and nonpigmenting lines were minus and plus, respectively. This sample is too small to warrant the conclusion that the pigmenting factor is sex-specific in this race.

Stability of pigmentation phenomenon

The stability of the pigmentation varied, depending on the race involved. It was strikingly stable in isolates from race T-16, as shown by the fact that the pigmenting lines became darker with increasing age while the nonpigmenting lines remained essentially colorless. Only an occasional isolate among the nonpigmenting lines showed a tendency to pigment in old cultures. These lines, however, seldom were as intensely brown as the true pigmenting lines.

Isolates of race T-10 were the most unstable with regard to pigmentation. In this race the pigment was slower to develop and was less intense than in T-16. Consequently, older cultures of the T-10 isolates were required for separating the lines into pigmenting and nonpigmenting groups. Beyond a certain age, however, many of the lines that were classified originally as colorless tended to develop a dull brown color, often becoming indistinguishable from true pigmenting lines.

Some tendency toward instability of pigmentation was evident in the lines from T-15. Usually these lines were easily separable into pigmenting and nonpigmenting groups after two weeks. However, after 20 days, the nonpigmenting lines began to darken sufficiently to be confused with pigmenting lines.

Summary and Conclusions

An association between pigment production in agar culture and sex compatibility in *Tilletia caries* was observed first in a set of monosporidial lines of race T-16. Compatibility, as determined by sporidial fusion in culture, showed that the pigmenting and nonpigmenting lines were of opposite sex. This association between sex-compatibility and pigmentation occurs also in races T-10 and T-15. There was no such association in two sets of monosporidial lines of race T-9.

Expression of pigmentation differences in the various sets of monosporidial lines was intensified on potato-sucrose-agar.

Association between pigmentation and sex-compatibility, where

it occurred, was not sex specific, since sets of pigmenting lines sometimes were of opposite sex.

Pigmentation was most stable in race T-16, least stable in race T-10, and showed some instability in race T-15.

Pigmentation of the culture medium may have potentialities as a technique in genetic studies of the bunt fungi where large numbers of compatibility relationships must be determined. As shown by this study, however, there is some evidence that pigmentation and compatibility are not associated in all races of *T. caries*. This inconsistency may limit its usefulness to certain races. In this connection temperature may be important. Preliminary studies indicate that temperature has a pronounced effect upon the pigmentation phenomenon.

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THE NATURE AND EXTENT OF MERCURY INJURY AND ASSOCIATED ANATOMICAL RESPONSES IN WHEAT SEEDLINGS*

LAURENCE H. PURDY†

INTRODUCTION

All mercury preparations used as seed treatment fungicides against wheat smut [*Tilletia caries* (DC.) Tul. and *T. foetida* (Wallr.) Liro] in the Pacific Northwest are more or less phytotoxic, depending on rate of application. Consequently, injury to seed and seedlings sometimes accompanies seed treatment with these materials, the degree of such injury increasing in proportion to increases in the rates of application. Thus, in a previous study (6) it was shown that three mercury fungicides (Panogen, Ceresan M, and Agrox) produced little or no injury at the recommended rates of application (three-fourths ounce per bushel for Panogen and one-half ounce for Ceresan M and Agrox) whereas significant injury occurred at rates in excess of one ounce. This injury was expressed as inhibition and retardation of emergence and as hypertrophy of the plumule and primary roots (7). Similar symptoms of mercury injury to wheat seedlings were reported by Crosier (1) but neither of the above reports dealt with the anatomical nature of the hypertrophy.

Various types of injury to several crops caused by mercury and other chemicals have been reported (2, 3, 5, 9), and Sass (8) found that mercury injury to corn seedlings was expressed as hypertrophy of the plumule and roots without an increase in cell numbers. The purpose of the study reported here was to determine the nature of the anatomical changes occurring in wheat seedlings injured by mercury.

MATERIALS AND METHODS

Seed of the winter wheat varieties Brevor, Omar, and Rio and the spring variety Red Bobs was treated with Ceresan M (7.7 per-

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cent N-(ethylmercuri)-p-toluenesulfonanilide) at the one-half ounce rate recommended for bunt control and also at one and two ounces per bushel. Some of the treated seed was germinated in the laboratory on moist filter paper in Petri plates and some was planted in the greenhouse. Germination percentages were determined in the Seed Testing Laboratory of the State College of Washington.[1]

The degree of toxicity was recorded as reductions in germination and seedling growth, as reflected by the length of the coleoptile and roots of four-day-old seedlings. For this study germination was defined as the production of a seedling free from deformities and with an apparent potential to develop into a normal mature plant.

For the study of anatomical responses the injured and non-injured seedlings to be sectioned were killed and fixed in Randolph's modified Navashin fluid (4) for 12 to 24 hours and then washed in running water for four hours prior to dehydration in the standard ethyl alcohol-butyl alcohol series. The dehydrated tissues were embedded in Tissuemat[2] and sectioned at 7 to 10 microns. The mounted sections were stained according to Conant's quadruple stain schedule (4).

Results

The degree of injury caused by Ceresan M at different rates of application to four wheat varieties is indicated by the germination percentages shown in Table I. A significant reduction in germination occurred in all varieties treated at rates of one ounce or more per bushel. There was also good evidence of differential tolerance to the phytotoxic properties of the fungicide among the four varieties tested. Red Bobs and Rio were more tolerant than Brevor and

TABLE I. Germination Percentages of Four Wheat Varieties Treated with Ceresan M at Three Rates of Application

Treatment	Ounces per Bushel	Wheat Variety and Percent Germination			
		Red Bobs	Brevor	Omar	Rio
Check		98	98	97	94
Ceresan M	½	98	100	95	94
Ceresan M	1	91	54	43	65
Ceresan M	2	33	2	0	14

[1] Grateful acknowledgment is made to A. E. Overland for determining the percentage germination in the laboratory.

[2] A paraffin embedding compound supplied by the Fisher Scientific Company.

Omar, especially at the one- and two-ounce rates. Treatment rates in excess of one ounce nearly destroyed the seed viability of all four varieties.

The root and coleoptile lengths following different treatment rates are summarized in Table II. These show clearly that early development of the seedlings was markedly influenced by the treatments. At the one-half-ounce rate the roots and coleoptiles were longer than those from untreated seed, especially in the variety Red Bobs. At the higher rates the treatments were clearly detrimental, as indicated by the greatly reduced seedling development (Table II).

TABLE II. ROOT AND COLEOPTILE LENGTH IN MILLIMETERS OF FOUR-DAY-OLD WHEAT SEEDLINGS GROWN FROM SEED TREATED WITH CERESAN M AT DIFFERENT RATES OF APPLICATION

		Wheat Variety; Root (r) & Coleoptile (c) Length							
	Ounces per	Red Bobs		Brevor		Omar		Rio	
Treatment	Bushel	r	c	r	c	r	c	r	c
Check		22	6	19	6	19	5	23	8
Ceresan M	½	32	9	20	6	23	7	24	10
Ceresan M	1	12	5	11	4	13	7	15	8
Ceresan M	2	13	5	3	3	3	3	9	5

The mildest form of injury which occurred at the one-ounce rate was a general stunting of the seedlings (Fig. 1B) and enlargement of the roots immediately behind the apex. Although the growth of the first three primary roots usually was entirely suppressed, the mildly injured seedlings were able to resume normal growth and develop mature plants after formation of the fourth and fifth primary roots (Fig. 1G).

In seedlings severely injured by high rates of application, the coleoptiles were shortened and thickened, becoming almost spherical (Fig. 1D & E). Complete inhibition of germination occurred to some extent in all varieties at high rates of application.

The anatomical responses associated with mercury injury to seedlings and roots are illustrated in Figures 2, 3, and 4. Longitudinal and transverse sections of four-day-old uninjured and injured seedlings are shown in Figure 2. The shortened and thickened coleoptile and plumule of a mercury-injured seedling (Fig. 2B) are shown in contrast with those of a normal seedling (Fig. 2A). Reduction in length of these structures was accompanied

by a thickening of their tissues in general, as reflected in the larger diameter of the injured seedling (Fig. 2C).

The nature of the general hypertrophy of the injured seedling was due to an increase in cell size rather than in cell number. This is shown in Figure 3, where enlarged longitudinal and transverse sections show larger and fewer cells in the injured than in the uninjured seedlings. The cell nuclei also were greatly enlarged, especially in the meristematic tissues of the plumule and roots. There were no multinucleate cells, such as those observed by Sass (8) in mercury-injured corn seedlings. Vacuolization of cells occurred closer to the shoot apex in the injured than in the uninjured seedlings. No abnormal thickening of the walls of the enlarged cells of the coleoptile was evident.

The abnormal enlargement of mercury-injured roots immediately behind the apex is illustrated in Figure 4. In the region of the root initials the cells were only slightly enlarged, whereas outside this area the cells were greatly enlarged. As in the plumule, enlarged nuclei were also present in the root cells, especially in the meristematic tissues. In contrast to the plumule, the cell walls of injured roots were somewhat thickened, especially in the xylem and phloem and occasionally in the vascular parenchyma.

Summary

Seed of four varieties of wheat was treated with Ceresan M at three rates (½, 1, and 2 ounces per bushel). Germination and seedling growth were compared with that from untreated seed. Seed treatment at rates of one or more ounces per bushel reduced germination and altered seedling development. Treatment with one-half ounce per bushel did not reduce germination and produced larger seedlings than untreated seed. Injury to germination and growth was greatest at the highest rate of application, ranging from mildly arrested development to severe hypertrophy of the roots and coleoptile. The hypertrophy of injured seedlings was due to an increase in cell size rather than to an increase in cell number. The cell nuclei also were enlarged, especially in meristematic tissues.

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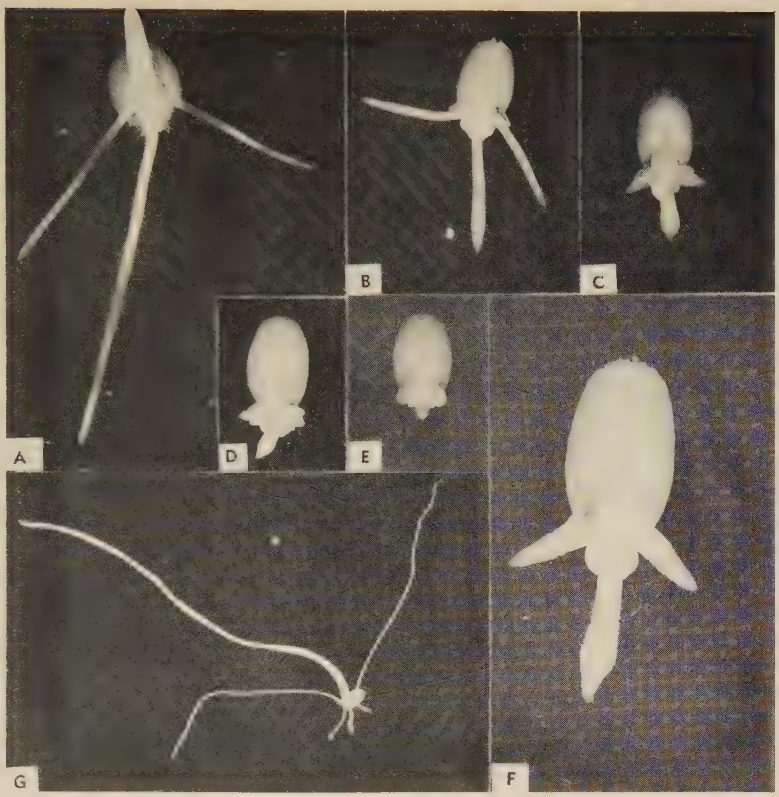

Figure 1. Seedlings of Red Bobs wheat illustrating various degrees of mercury injury: (A) healthy; (B) mildly injured; (C, D, and E) severely injured; (F) enlarged view of severely injured seedling; (G) mildly injured seedling showing recovery as indicated by the development of the fourth and fifth primary roots.

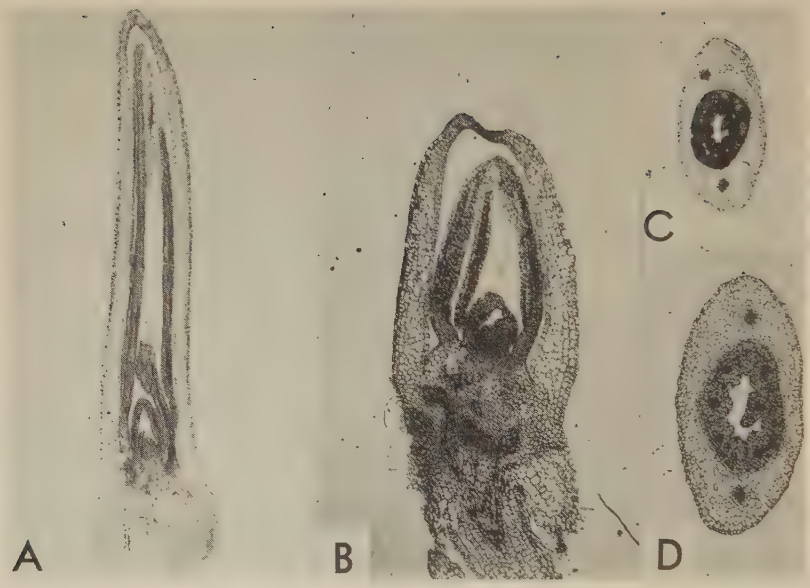

Figure 2. Photomicrographs of longitudinal (A and B) and transverse (C and D) sections of healthy and mercury-injured wheat seedlings showing nature of injury as reflected in tissue development. A and C healthy; B and D injured. (16x)

Figure 3. Transverse (A and B) and longitudinal (C and D) sections showing differential size of cells of healthy (A and C) and mercury-injured (B and D) seedlings. Size difference of cells in healthy and injured seedlings is particularly noticeable by contrasting A and B or C and D. (50x)

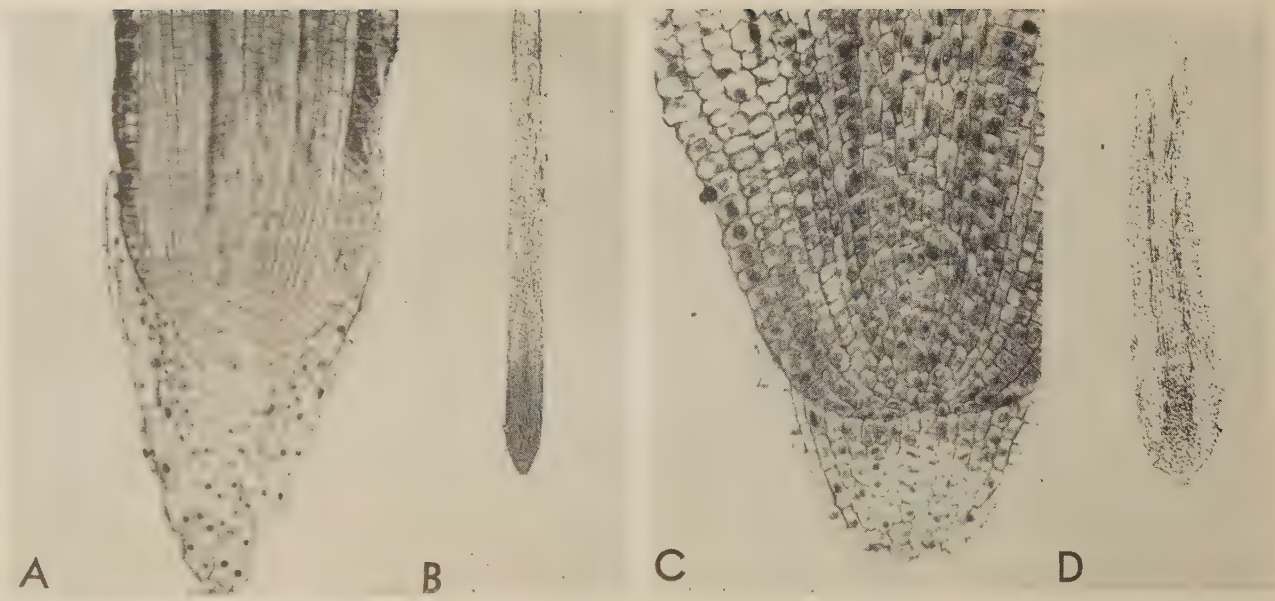

Figure 4. Longitudinal sections of healthy (A and B) and mercury-injured (C and D) roots of wheat seedlings showing nature of hypertrophy of roots. Cell size differences between healthy (A) and injured (C) roots are apparent even at the root apex. A and C (66x); B and D (14x).

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INHERITANCE OF PATHOGENICITY IN *USTILAGO AVENAE**

P. M. Halisky†

Introduction

Loose smut of oats, caused by *Ustilago avenae* (Pers.) Rostr., is world-wide in its distribution and probably is coextensive with the cultivated oat crop. It has been reported from sixty-one countries, in some of which this disease constitutes a major hazard to economic oat production (8). In the United States, for example, estimates show that in some years smut destroys forty to fifty million bushels of oats, the heaviest losses occurring in the South Atlantic States. Annual losses up to 35 per cent have been reported from some states of this region (33). Here, as elsewhere, *U. avenae* is the predominant species, being much more prevalent than *Ustilago kolleri* Wille. This predominance reflects, at least to some extent, the dynamic potential of the loose smut species, a potential that is further symbolized by its pathogenic diversity. Every study of pathogenic specialization in the oat smuts has shown a much greater degree of specialization in *U. avenae* than in *U. kolleri* (32, 40).

Aside from its economic implications with respect to oat production, pathogenic specialization in the oat smuts greatly complicates the problem of breeding of new varieties of oats. Since varieties react differently to the various pathogenic races, a breeding program must be aimed at the production of varieties with resistance to as many different races as possible. Even then such a variety might exhibit high susceptibility in commercial production, owing to the advent of new or previously unknown pathogenic races. In practical experience such new races are encountered frequently, especially in *U. avenae* (29). A notable example is the

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past experience with Victoria, an introduced variety of the species *Avena byzantina* C. Koch. The wide use of this smut- and rust-resistant variety in the oat-breeding programs throughout the nation resulted in the release of about thirty named varieties of oats containing Victoria germ plasm (42). The subsequent appearance of new races of *U. avenae* pathogenic to this type of germ plasm rendered all of these varieties susceptible and thereby caused serious setbacks in the organized efforts of plant breeders and plant pathologists to control this disease by growing resistant varieties. Today these pathogenic races are widespread throughout the oat-growing regions of the United States.

Systematic studies on genetic relationships in the smut fungi apparently began after Kniep (35) demonstrated in 1919 that sporidial fusion in *Ustilago violacea* (Pers.) Roussel occurred only between certain sporidia. The experimental production of hybrids between physiologic races in smut fungi was demonstrated in 1928 by Goldschmidt (10), who obtained evidence that differences in pathogenicity in *U. violacea* were controlled by single Mendelian genes.

The heritability of factors governing pathogenicity in the bunt fungi has been conclusively demonstrated by Holton (17, 25, 28). Pathogenicity in physiologic races of these fungi is controlled by one or more genetic factors, and physiologic races may be either homozygous or heterozygous for pathogenicity. Inbred lines of homozygous races resemble the parent race, while those of heterozygous races exhibit wide ranges in pathogenicity.

The existence of a multiplicity of pathogenic races of *U. avenae* undoubtedly is contingent on the expression of a variety of genetic factors. The pathogenic stability of such races is evident in their ability to retain distinct differential reactions through repeated propagation on standard host tester varieties. Furthermore, as indicated above, the genetic basis of pathogenicity has been established in several species of smuts, and new pathogenic types can be produced by crosses between species and between races within a species. In the oat smuts, for example, the heritable nature of pathogenicity factors is well established, but the number of factors involved and the mechanism of their heritability have not been adequately explained. For instance, why, genetically, does one race (A5) infect only four varieties, another race (A6) these same four and one additional variety, while a third race (A7) is pathogenic on these

same five varieties and on one additional variety as well? What governs the distinct pathogenic bounds of these specific races and how, genetically, do they differ from each other? The fact that these races have maintained their distinct pathogenic identity in annual inoculation studies for more than a decade is evidence of underlying genetic control of pathogenicity. The need to elucidate the fundamental nature and number of factors governing pathogenicity in *U. avenae* prompted the investigation of this heritable character.

Review of Literature

The discovery of spore germination in *Tilletia caries* (DC.) Tul. and *Ustilago carbo* DC. in 1807 by Prevost, paved the way for extensive investigations on the general biology of the whole group of smut fungi. Throughout the latter half of the nineteenth century extensive and thorough studies of the smut fungi were made by a number of investigators, notably Tulasne, DeBary, Fischer von Waldheim, Brefeld, Dangeard, and Harper. Their carefully recorded and detailed observations form the basis of our present concept of the fundamental nature of these organisms, and are now regarded as classics in mycological literature. While all of these workers observed fusions between sporidia and between promycelial cells, it was DeBary who first suggested the sexual nature of such fusions. He substantiated this view with the observation that the fusions were usually between two, and only two, sporidia (14).

The gametic nature of smut sporidia was first discovered by Kniep (35) when he demonstrated that sporidial fusion in *U. violacea* (Pers.) Roussel occurred only between certain sporidia. This discovery of heterothallism in smuts led to the successful application of modern genetic principles to the study of this group. Thus was laid the foundation for studies of hybridization and mutation in the *Ustilaginales*. Kämmerling (34), in studies with hybrids between *U. longissima* (Schlecht.) Meyen and its variety *'macrospora'*, demonstrated the heritability of smut spore characters, the nature of spore germination, and sporidial production. This pioneer work on hybridization and genetic studies in these smuts formed the basis for later investigations of genetics in other smut fungi.

Studies on hybrids between a few closely-related species of smuts have shown certain spore and sorus characters are inherited along Mendelian lines. In the oat smuts, for example, Holton (18, 19, 21) and Popp and Hanna (39) found spore echinulation dominant

in crosses between *U. avenae* and *U. kolleri.* Allison (1) and Bever (4) found that spore echinulation was also dominant in crosses between the barley smuts, *U. hordei* (Pers.) Lagerh. and *U. nigra* Tapke. Inheritance studies of sorus characters in oat smuts revealed that the loose type of smutted panicle was dominant over the covered type, according to Fischer and Holton (9) and to Hanna and Popp (15). Another type of heritable sorus produced by a mutant of *U. avenae* was also described by Holton (24). Unlike the usual brown, powdery type, a distinctly black indurate sorus was found on oats. According to Holton the factor for powdery sorus was dominant over the factor for the indurate sorus type. Segregation and recombination of factors for these characters occurred in a 3:1 ration indicating simple monohybrid inheritance.

An unusual type of sorus was also described by Reed and Stanton (41) on hybrids of Markton oats. The host inflorescence appeared as rudimentary panicles with sterile spikelets that resembled the common symptoms of blasting. When oat hybrids, of which Markton was one of the parents, were inoculated with *U. avenae* such panicles appeared rather commonly although no spores were produced. All circumstances surrounding this phenomenon indicated that the sterility was due to the infection of these plants by the smut fungus. A similar type of infection manifestation on the variety Camas is characteristic of one of the races (A7) used in this study on inheritance of pathogenicity. Its genetic implications were therefore included as part of the study.

As previously indicated, the existence of a multiplicity of physiologic races within the species of smut fungi indicates the complexity of the problem of inheritance of pathogenicity. A number of investigators have studied the inheritance of pathogenicity in the smuts. From their studies it is apparent that pathogenicity is a heritable quality. However, the exact nature of this inheritance in the oat smuts has not been determined (27).

In a cross between the wheat smut species, *Tilletia caries* and *T. foetida* (Wallr.) Liro, Holton (23) obtained a new, pathogenically distinct race. Later, Holton (25) also crossed two races of *T. caries* and obtained a distinct hybrid race with greater virulence than either parent race, thus indicating that pathogenicity could be inherited transgressively. Allison (1) and Bever (4) also obtained segregates from hybrids between *U. hordei* and *U. nigra* which produced higher percentages of infection on barley than did either parent.

In 1928 Goldschmidt (10) observed that the matings of particular monosporidial lines of *U. violacea* influenced profoundly their parasitic reaction. Goldschmidt studied the pathogenicity of experimentally produced hybrids between six biologic strains of anther smut and obtained evidence that differences in pathogenic specialization were based on a single Mendelian gene. Similar evidence of a simple monogenic basis for differences in pathogenicity between certain race hybrids of *U. avenae* was reported by Halisky and Holton (13).

In the smut fungi an individual spore from a collection or race may be heterozygous for pathogenicity factors. This was clearly shown by Nicolaisen (38), who obtained infection on some selected varieties of oats by inoculating them with paired monosporidial lines of *U. avenae*. He demonstrated that two sporidial matings from a given spore produced 100 per cent smut on the variety Lischower while two other matings from the same spore gave negative results on this variety. Nicolaisen (38) also showed that factors for pathogenicity in the nucleus of one of the two monosporidial lines making up the dicaryophyte may be dominant on one variety, recessive on another, and mixed dominant and recessive on a third variety. In crosses between sporidia representing races of known pathogenicity Halisky (12) reported incomplete dominance of factors governing pathogenicity in certain race hybrids of *U. avenae*.

Holton (22, 26) also obtained evidence of heterozygosity of factors governing pathogenicity in some races of *U. avenae*. The inconsistent high and low infection produced by different inbred lines from the same spore indicated the existence of heterozygosity of factors for pathogenicity. Further evidence of this was obtained when monosporidial lines from two races were combined. The pathogenicity of the monosporidial combinations representing crosses between races was either very high or contrastingly low, suggesting an interaction of complementary factors for pathogenicity. Christensen (5) also found that a monosporidial line of *U. zeae* may be strongly pathogenic in combinations with certain lines of opposite sex and only weakly pathogenic with other lines of opposite sex. It seemed clear that different lines had different factors for pathogenicity apart from those for sex. Christensen (5, 6) concluded that in *U. zeae* there were multiple factors for pathogenicity and that these were independent of those governing sporidial fusion.

The heritability of pathogenicity factors in the smut fungi is

well established, and new combinations give rise to new kinds of pathogenicity. In crosses of smuts with well-defined pathogenicity, segregates have been isolated which differ in pathogenicity from both parents. By crossing *U. avenae* and *U. levis* (Kellerm. and Sw.) Magn., Holton (20) produced a synthetic strain of *U. levis* that was more widely virulent that either parent. He also produced a strain of buff smut which differed pathogenically from both its *U. avenae* and buff smut parents. By this experimental production of new pathogenic races Holton demonstrated how hybridization between species may play an important role in the development of new pathogenic strains of oat smut fungi.

The inheritance of factors governing pathogenicity in the oat smut fungi has been shown in some studies to be independent of other characters. Thus Holton (24) concluded that the pathogenicity of any race of *U. avenae* or *U. kolleri* could be transferred to the buff race by hybridization. Segregation and recombination of factors for sex, pathogenicity and spore color were independent. Therefore it is possible to select for any one of several possible types in the segregating generation. Similar evidence was obtained by Utter (44) from an inter-species hybrid of *U. avenae* and *U. levis*. He showed in this study of experimentally produced races that the factors for pathogenicity and gross morphology of the diseased plant were inherited independently.

In a study of pathogenicity in *U. avenae,* Leitzke (36) inoculated ten varieties of oats with spore mixtures of six pathogenic races and obtained divergent results. The high degree of variability was attributed to hybridization occurring between the races. Leitzke also obtained a distinct race which presumably originated in his experiment by hybridization within the host. Meiners (37) pointed out that smut spores represent the diploid phase and cannot be propagated independently. When the spores germinate, reduction division and segregation of various factors take place. As a consequence, each new generation may consist of a new group of related biotypes, which may give differential reactions of pathogenicity.

The potentialities of hybridization between *U. avenae* and *U. kolleri* and their respective races have been indicated by the experimental results of Holton (20) and Utter (44). In practical experience, new races of *U. avenae* are encountered frequently according to Hansing (16), Holton (29), and Holton and co-workers (30, 31).

This suggests sufficient intermingling of distinct types to create a dynamic potentiality with respect to pathogenicity in this oat smut fungus. The origin of new pathogenic types from combinations of known types is probably the primary factor in the perpetuation of the smut problem in oats. Hence the importance of investigating the nature of inheritance of factors for pathogenicity in *U. avenae.*

Materials and Methods

The adoption of a standard system of race identification in the oat smuts was proposed by Holton and Rodenhiser (32). They described 15 pathogenic races of *U. avenae.* These races, designated as A1 to A15, were differentiated by their reaction on ten standard host varieties. Subsequent work by Hansing (16) resulted in the description of five additional races (A16 to A20) of this pathogen. The comparative reactions of all these races are summarized in Figure 1. From these twenty pathogenic races of *U. avenae,* three (A5, A6, A7) were selected for this study. As shown in Figures 1 and 2, races A5 and A6 are differentiated by their reactions on Monarch, while A6 and A7 differ in pathogenicity on Camas.

The selection of races which are thus closely related genetically and yet distinction in pathogenicity is an important consideration. Induced hybridization studies involving interracial crosses in species of *Ustilago* may result in sterility and failure of hybrid monosporidial lines to grow. This hybrid sterility in intra-specific crosses has been observed by Meiners (37) and by Holton (unpublished data). To circumvent this problem of probable sterility in smut hybrids, races A5, A6, and A7 were chosen as representative of gentically compatible yet discrete entities of *U. avenae* for hybridization studies. The consistent pathogenicity of these three races is well established from consecutive annual tests at Pullman, Washington, for more than a decade.

The differential varieties of oats used in these studies were the same as those used by Holton and Rodenhiser (32). The seed was grown in isolation plots and made available annually from stock supplies maintained in the Regional Smut Research Laboratory at Pullman. The three races (A5, A6, A7) used in this study are separable by their pathogenic reactions on six host varieties, as shown in Figure 2. These differential varieties (Anthony, Black Diamond, Victory, Gothland, Monarch and Camas) are cultivated varieties of the common oat species, *Avena sativa* L. A detailed

PATHOGENIC RACES OF *USTILAGO AVENAE*

RACES.	A	BD	V	G	M	F	BM	C	N	L
A1	S	S	S	R	R	R	R	R	R	R
A2	S	S	S	R	R	R	R	S	R	R
A3	S	S	S	I	S	R	R	R	R	R
A4	S	S	S	R	S	R	S	R	R	R
A5	S	S	S	S	R	R	R	R	R	R
A6	S	S	S	S	S	R	R	R	R	R
A7	S	S	S	S	S	R	R	S	I	R
A8	S	S	R	R	R	R	R	R	R	R
A9	S	S	R	R	R	S	R	R	R	R
A10	S	R	S	S	R	R	R	R	R	R
A11	S	R	S	S	R	R	R	R	S	R
A12	R	S	R	I	R	S	R	R	R	R
A13	R	R	R	R	S	R	R	R	R	R
A14	S	S	I	R	R	S	R	R	R	S
A15	R	S	R	S	S	S	R	R	R	S
A16	S	S	S	S	R	S	R	R	R	R
A17	R	S	R	S	S	S	R	R	R	R
A18	R	S	R	S	R	S	R	R	R	R
A19	S	S	S	S	S	R	R	R	I	R
A20	S	S	S	S	S	R	R	R	R	S

Figure 1. Race-reaction chart showing twenty pathogenic races of *Ustilago avenae* differentiated on ten varieties of oats. The differential varieties are designated by letters as follows: A—Anthony, BD—Black Diamond, V—Victory, G—Gothland, M—Monarch, F—Fulghum, BM—Black Mesdag, C—Camas, N—Nicol, and L—Lelina. The races are designated as A1 to A20 and the varietal reactions are indicated as R—Resistant, I—Intermediate, and S—Susceptible.

REACTION OF THREE RACES OF USTILAGO AVENAE ON SIX VARIETIES OF OATS

RACES	DIFFERENTIAL VARIETIES					
	A	BD	V	G	M	C
A5	S	S	S	S	R	R
A6	S	S	S	S	S	R
A7	S	S	S	S	S	S

S=SUSCEPTIBLE R = RESISTANT

Figure 2. Chart showing the pathogenic reactions of three parent races of *Ustilago avenae* on six differential varieties of oats.

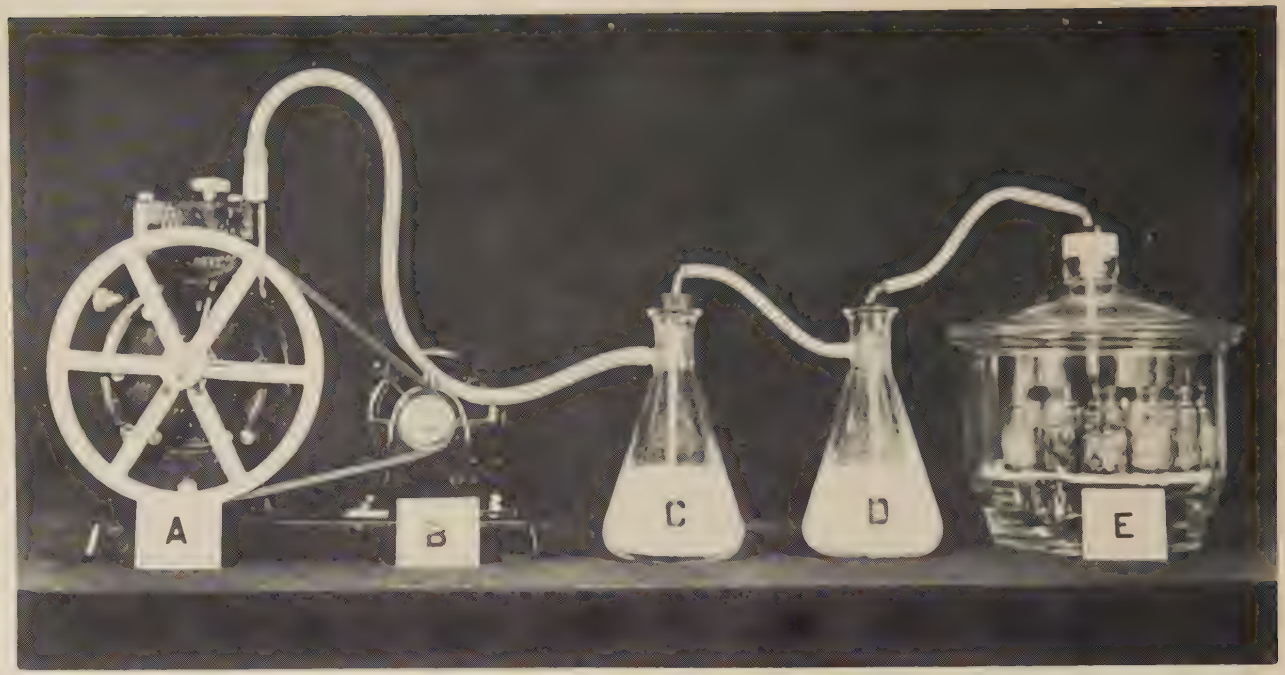

Figure 3. Photograph showing the equipment used in the partial-vacuum method of inoculating oat seed with *Ustilago avenae*. A—Vacuum Pump. B—Electric Motor. C and D—Calcium Chloride Drying Agent. E—Vacuum Desiccator with Screw-Cap Jars Containing Seed and Water Suspension of Spores.

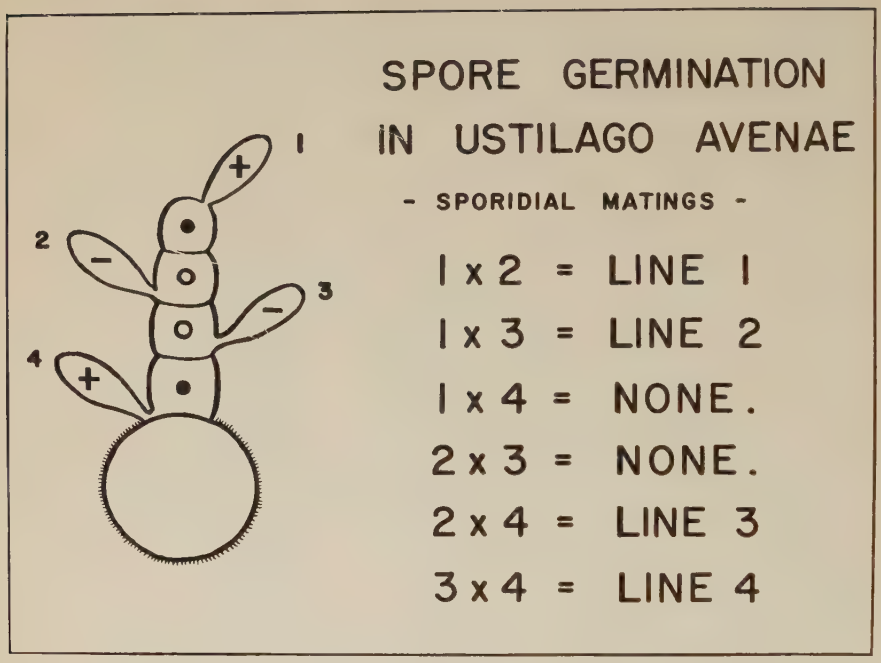

Figure 4. Diagram showing pattern of mating sporidia used in the production of four selfed lines from each spore.

		RACE - A7 SPORIDIUM NUMBER			
		71	74	72	73
RACE A6 SPORIDIUM NUMBER	61	+	+	−	−
	63	+	+	−	−
	62	−	−	+	+
	64	−	−	+	+

Figure 5. Chart showing the results of sixteen possible matings between the monosporidial lines from two parent spores of *Ustilago avenae.* (The plus signs (+) here indicate compatibility whereas the minus signs (—) indicate lack of fusion between paired sporidia.)

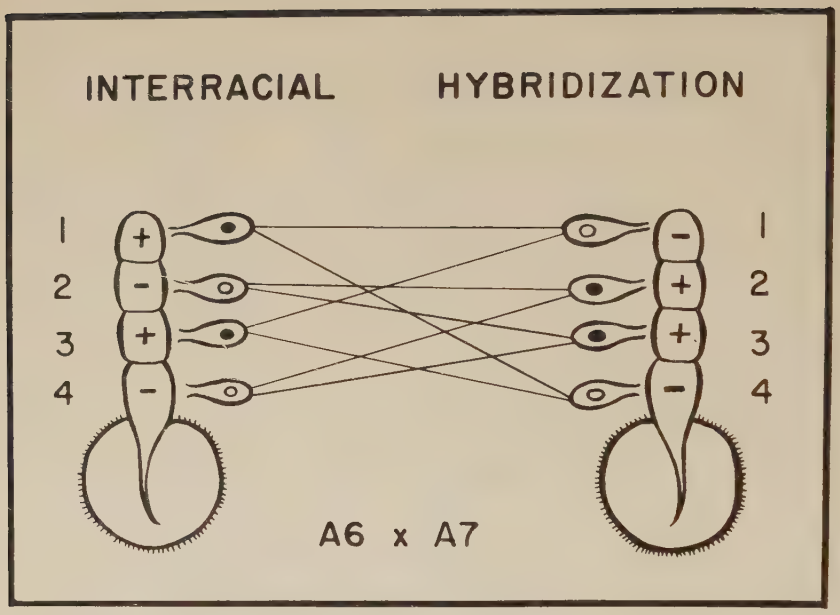

Figure 6. Diagram showing the origin of eight compatible combinations of sporidia of *Ustilago avenae* representing an interracial cross.

Figure 7. *Ustilago avenae* on Monarch oats showing sporulation at the base of each spikelet. (Infection in the black-hulled varieties, Monarch and Black Diamond, was typically characterized by a covered sorus symptom type.)

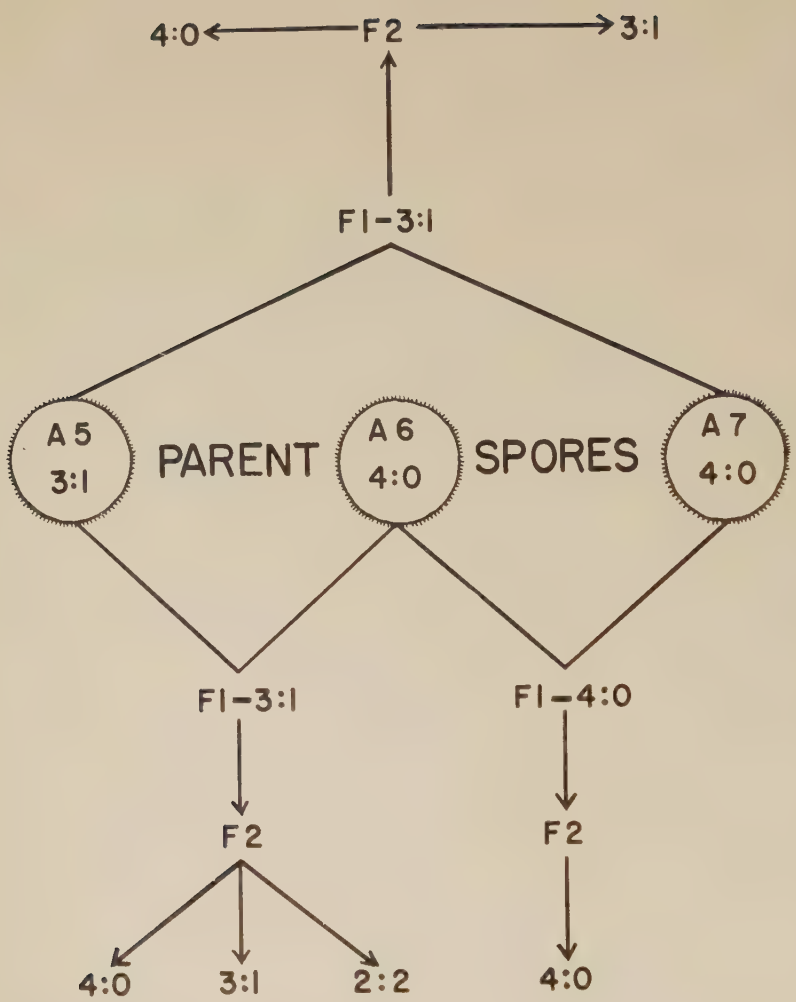

Figure 8. Diagram showing the origin of pathogenicity ratios characterizing the hybrid F_1 and F_2 progeny of interracial crosses.

description of each variety, except Camas, is found in the monograph on Oat Identification and Classification by Stanton (43). According to Coffman *et al.* (7), Camas originated from a cross between Markton and Victory.

Before inoculation, seed of the differential varieties was freed from natural contamination with smut spores or mycelium by soaking it in a dilute solution of commercial formalin (1 part formalin to 320 parts water) for ten to twenty minutes. After treatment, the seed was washed in running water and then spread out to dry.

The partial-vacuum method as described by Allison (1) was used almost exclusively for inoculating the seed. Figure 3 is a photograph of the equipment used in these studies. The seed was immersed in an aqueous suspension of inoculum in screw-cap glass vials. These vials, with the tops screwed on loosely, were then placed in a vacuum desiccator and subjected to partial vacuum for twenty minutes. The release of the vacuum was made abruptly to ensure the penetration of inoculum beneath the lemma and palea of each oat kernel. After settling, the excess water was poured off and the inoculated seed was dried on clean paper towels. The dried seed was then packaged and stored under cool, dry conditions until planting time. Under favorable conditions 80 to 100 per cent smut was produced in susceptible varieties.

The aqueous suspensions of inoculum used in these studies consisted either of smut spores or compatible combinations of two monosporidial lines. When spore inoculum was employed, the preparation of appropriate suspensions was greatly facilitated by the use of a Waring blendor advocated for such purposes in plant pathology research by Andrus (2). Entire panicles containing smut sori in the spikelets were immersed in distilled water and subsequently fragmented by the cutting blades. This suspension was immediately poured over the seed in the inoculating vials. After each inoculum preparation the blendor was sterilized with 70 per cent ethyl alcohol and then thoroughly rinsed in running water.

In this study monosporidial cultures were used for hybridization. Complete sets of such cultures were obtained from each germinating spore by isolating the four primary sporidia with the aid of a Chambers micromanipulator. Thus a set of monosporidial lines consisted of four cultures, these being representative of all the end-products of meiosis. The first-formed sporidium from each cell of the promycelium was isolated and numerically identified accord-

ing to its origin on the promycelium as one to four, beginning at the apex (Fig. 4). The monosporidial isolations were then incubated at room temperature until the sporidia had multiplied to a desired quantity on nutrient-agar medium. The compatibility group of each line was determined by using the Bauch test developed specifically for this purpose (3). From four monosporidial lines mated in all possible combinations, four compatible pairings were identified, as outlined in Figure 4. These constituted the selfed progeny from any given spore. The production of vigorous, white *suchfaden* by the compatible combinations of fresh, rapidly-growing sporidial cultures made possible the use of the Bauch test for determining the compatibility groups among haploid lines of *U. avenae.*

In cross-mating sporidia isolated from different promycelia, the Bauch test was again employed to determine the compatible combinations. In these crosses the race number was inserted before the sporidium number for identification. Thus 61 × 73 means sporidium-1 from race A6 was mated with spordium-3 from race A7. When the four monosporidial lines from one spore were mated with the four monosporidial lines from another spore, sixteen combinations were possible, as shown in Figure 5. However, judging by the production of the conspicuous aerial hyphae which indicate compatibility according to the Bauch test, only one-half or eight such combinations were potentially infective. This, of course, was contingent on the distribution of the compatibility groups, as is evident from Figure 6. Thus in any out-crossing experiment, such as interracial hybridization, eight pathogenic combinations form the hybrid F_1 population.

Inoculated seed of the host varieties was planted either in greenhouse benches or in field plots. Inoculum from each crop was collected early just as the smutted panicles were emerging from the boot. By collecting these panicles while they were still enveloped by the sheath, contamination by wind-borne spores from other smutted plants in the vicinity was reduced to a minimum. Spore inoculum of this type was stored under refrigeration for future use. Large plots of inoculated varieties were usually grown in the field each summer where counting was delayed until all the panicles had emerged. Stands of two hundred to four hundred culms per three-foot row were usually obtained. Infection counts were made on a panicle basis and infection percentages were subsequently computed to determine the degree of infection.

Experimental Results

Characterization of the parent races employed in these studies

Throughout this study the pathogenic reactions of the three parent races, A5, A6, A7 of *Ustilago avenae,* were separately checked each year. Smutted oat panicles representing each race were collected from the test plots each summer and held for use the following year. These tests indicated the reactions of the parent races for comparison with those of the hybrid lines; thus they served as parental controls. The reactions obtained with each race during four consecutive years are indicated by the data in Table I. According to these infection percentages the pathogenicity of each race was consistent from year to year. It is apparent that these selected races are relatively stable pathogenically, which is a desirable feature in a genetic study of this specific character. As shown in Table I the stability of the parental races is indicated by the relatively high

TABLE I. The Pathogenic Reactions of Parent Races A5, A6 and A7 of Ustilago avenae on Six Varieties of Oats During Four Consecutive Annual Trials

Race	Year	Percentage of Infection[a]					
		Anthony	Black Diamond	Victory	Gothland	Monarch	Camas
A5	1953	91	77	81	85	0	0
	1954	93	81	95	91	0	0
	1955	86	67	77	86	0	0
	1956	82	50	67	66	0	0
	Av.	88	69	80	82	0	0
A6	1953	75	47	51	64	57	0
	1954	88	68	55	72	76	0
	1955	65	68	59	64	88	0
	1956	72	54	47	62	80	0
	Av.	75	59	53	66	75	0
A7	1953	88	80	75	92	95	70
	1954	88	64	62	78	94	20
	1955	70	71	51	79	88	66
	1956	65	60	57	70	86	55
	Av.	78	69	61	80	91	53

[a] Percentages of infection computed on a panicle basis.

infection percentages (47 to 95 per cent) obtained on the susceptible varieties Anthony, Black Diamond, Victory and Gothland. Race A5 is characterized by a complete lack of infection on Monarch and Camas, whereas A7 produces consistently high infections on both of these varieties each year. Race A6, on the other hand, produces infection on Monarch but not on Camas.

In studying the inheritance of pathogenicity in interracial hybrids of *U. avenae,* it was necessary to ascertain the degree of homozygosity or heterozygosity of the parent races. As already shown, the four sporidia formed on a promycelium represent the end-products of meiosis. They are haploid progeny and may be isolated and crossed. The production of selfed lines through compatible matings of plus and minus sporidia from the same promycelium is illustrated in Figure 4. The degree of homozygosity is established by testing the inbred lines of the differential varieties and comparing their pathogenic reactions with those of their respective parent races. Selfed lines from homozygous races resemble the parents in pathogenicity, while those of heterozygous races exhibit wide differences in pathogenicity. In this study each parent race, A5, A6, and A7, was selfed. The pathogenic reactions of the selfed lines are indicated by the infection percentages in Table II.

It is apparent from the data in Table II that each parental spore selected for selfing was characterized by a high degree of homozygosity for pathogenicity factors. This is indicated by the fact that the reactions of the selfed lines resemble those of their respective parent races included at the bottom of the table. Thus, each of the selfed lines from Spores 1 and 2 in Table II characteristically infected four varieties only as did the parent race A5. Similarly, each of the selfed lines from Spores 3 and 4 infected these same four varieties and in addition the variety Monarch. These reactions resemble those of A6, the parent race. Likewise, the pathogenicity expressed by the selfed lines from Spores 5 and 6 characteristically resemble those of A7, their parent race. This is indicated by the uniform infection of all six varieties by each of the selfed lines derived from Spores 5 and 6. However, since this study represents only a limited number of spores, the results do not preclude the possibility of a heterozygous condition of pathogenicity factors. Nevertheless, a high degree of homozygosity is suggested as the usual genotypic condition prevailing in these three races.

TABLE II. PATHOGENIC REACTIONS ON SIX HOST TESTER VARIETIES OF PROGENY OBTAINED BY SELFING PARENT RACES A5, A6, AND A7 OF USTILAGO AVENAE

Spore Number	Sporidial Matings	Percentage of Infection					
		Anthony	Black Diamond	Victory	Gothland	Monarch	Camas
1-A5	1×2	100	50	100	94	0	0
	1×3	96	100	87	88	0	0
	4×2	94	78	68	88	3[a]	0
	4×3	100	77	75	77	0	0
2-A5	1×2	83	89	79	84	0	0
	1×4	95	83	81	91	3[a]	0
	3×2	70	56	65	80	0	0
	3×4	88	80	91	80	0	0
3-A6	1×2	80	70	59	54	50	0
	1×4	50	56	60	47	56	0
	3×2	100	53	44	67	65	0
	3×4	57	39	40	47	73	0
4-A6	1×3	79	23	36	70	47	0
	1×4	80	65	41	78	45	0
	2×3	80	47	63	74	68	0
	2×4	75	21	63	74	50	0
5-A7	1×2	89	66	77	94	95	63
	1×4	81	79	78	91	96	63
	3×2	89	85	74	93	94	72
	3×4	85	81	67	94	96	71
6-A7	1×3	92	81	61	93	91	67
	1×4	91	77	84	91	100	66
	2×3	92	83	83	91	92	74
	2×4	82	86	75	91	96	81
A5 . . .	. . .[b]	88	69	80	82	0	0
A6 . . .	. . .	75	59	53	66	75	0
A7 . . .	. . .	78	69	61	80	91	53

[a] Infection probably due to seed mixture.
[b] Spore inoculum of parent races was used in growing the controls.

Pathogenicity of the interracial hybrid (F_1) progeny

The existence of sporidia of opposite compatibility groups derived from a single promycelium shows that *U. avenae* is heterothallic and amenable to genetic analysis. The significance of equal numbers of plus and minus sporidia is that all the haploid progeny

of meiosis may be recovered and utilized in a given cross. This means that the resulting genetic ratios are real, being mechanically rather than statistically determined. A complete cross between two parent races is constituted by eight separate compatible matings of sporidia, while the eight incompatible matings are not available for analysis. Thus a genetic evaluation of a hybrid (F_1) population from a cross between two races entails a comparison of the pathogenic reactions of eight hybrid lines out of 16 possible combinations. This is determined by the distribution of the plus and minus sporidia as indicated in Figures 5 and 6.

In this study crosses were made between the three races as follows: A5 $\times$ A6, A5 $\times$ A7 and A6 $\times$ A7. The pathogenicity expressed by the resultant hybrid lines is indicated by the infection percentages in Table III. A comparison of the four interracial crosses in this table reveals that the F_1 hybrids within a given cross may or may not express uniformity of infection. Thus in crosses 1 and 4 the pathogenic reactions of the hybrids were consistently high on all the susceptible varieties. These reactions are comparable to those of the respective parent races shown at the bottom of the table. This uniform or consistent infection of the differential varieties by these hybrids indicates a high degree of homozygosity for pathogenicity in the parents. On the other hand, there was evidence of heterozygosity in the parent races of crosses 2 and 3. Six of the eight hybrids in each of these crosses were strongly pathogenic; the remaining two hybrids of each cross (Nos. 9, 10, 17 and 18) were only weakly pathogenic. In these two crosses, therefore, there was segregation for pathogenicity in the ratio of 3:1 virulent to non-virulent lines, showing that the parent races were heterozygous for factors governing pathogenicity. It may be significant in this connection that sporidium No. 1 of the A-5 parent was common to all four hybrids exhibiting low virulence (Table III).

Interaction of factors for pathogenicity

The heterozygous state of factors governing pathogenicity in *U. avenae* shown by the data in Table III is best explained by the interaction of complementary factors. Furthermore, these data suggest that the expression of pathogenicity is not merely a summation of the separate effects produced by two genes alone. Pathogenicity is represented by an interaction of genes as shown when a given hybrid in crosses 2 and 3 is either highly pathogenic (62 to 100

TABLE III. Pathogenic Reactions of the Interracial Hybrid (F_1) Progeny of Ustilago avenae on Six Host Tester Varieties

Cross Number	Hybrid Number	Hybrid Combination[a]	Percentage of Infection					
			Anthony	Black Diamond	Victory	Gothland	Monarch	Camas
Cross 1 (A5xA6)	1	51 x 63	94	82	62	95	11	0
	2	51 x 64	86	71	71	96	7	0
	3	52 x 61	97	82	83	95	0	0
	4	52 x 62	93	50	57	85	13	0
	5	53 x 61	94	100	92	95	17	0
	6	53 x 62	100	84	91	83	24	0
	7	54 x 63	100	89	92	94	5	0
	8	54 x 64	100	88	78	87	5	0
Cross 2 (A5xA6)	9	51 x 63	1	3	1	----[b]	1	0
	10	51 x 64	8	5	7	----	1	0
	11	52 x 61	96	93	88	----	9	0
	12	52 x 62	100	91	90	----	5	0
	13	53 x 61	100	83	92	----	8	0
	14	53 x 62	100	89	89	----	7	0
	15	54 x 63	100	96	91	----	4	0
	16	54 x 64	100	99	91	----	4	0

(Cont.)

TABLE III—*(Continued)*

Cross Number	Hybrid Number	Hybrid Combination[a]	Percentage of Infection					
			Anthony	Black Diamond	Victory	Gothland	Monarch	Camas
Cross 3	17	51 x 72	8	5	7	4	3	0
(A5xA7)	18	51 x 74	2	1	1	1	2	0
	19	52 x 71	85	62	75	83	5	1
	20	52 x 73	95	81	87	92	7	1
	21	53 x 71	99	87	85	94	6	1
	22	53 x 73	97	91	86	92	6	2
	23	54 x 72	98	93	88	96	7	1
	24	54 x 74	99	93	86	95	5	2
Cross 4	25	61 x 72	95	92	86	87	97	0
(A6xA7)	26	61 x 74	94	88	86	84	92	0
	27	62 x 72	82	76	83	79	87	0
	28	62 x 74	74	74	72	68	88	0
	29	63 x 71	92	85	86	88	95	0
	30	63 x 73	95	89	87	87	94	0
	31	64 x 71	94	87	89	88	96	0
	32	64 x 73	91	81	85	89	91	0
A5	....	Spores	88	69	80	82	0	0
A6	....	Spores	75	59	53	66	75	0
A7	....	Spores	78	69	61	80	91	53

[a] The race number is inserted before the sporidium number.
[b] Seed of Gothland was not available for inoculation with Cross 2.

per cent infection) or relatively innocuous (1 to 8 per cent infection) on all the varieties. Furthermore, the low infections produced by the avirulent hybrids (Nos. 9, 10, 17 and 18) is not attributable to incompatibility of the monosporidial combinations. This possibility was ruled out by the use of the Bauch test, which clearly revealed the formation of vigorous fusion hyphae.

To explain pathogenicity in *U. avenae* on a genetic basis, the term "complementary factors" is applied. The term refers to two non-allelic genes that interact to produce a degree of pathogenicity different from that produced by either gene alone. It is postulated that only the interaction of two dominant non-allelic genes results in high infections on all susceptible varieties. If these two genes for pathogenicity are arbitrarily designated as A/a and B/b, then the nine possible genotypes may be simply arranged into two groups as follows:

(1) Strongly Pathogenic: AABB, AABb, AaBB, AaBb,

(2) Weakly Pathogenic: AAbb, Aabb, aaBB, aaBb, and aabb

A given sporidium which is gametic in nature is uninucleate and haploid and will not produce infection in this monocaryotic stage. The genetic constitution of a sporidium may thus be either AB, Ab, aB, or ab depending on the segregation of pathogenicity factors at meiosis. The fusion of two haploid sporidia marks the initiation of the dicaryotic or parasitic stage of the fungus. The parasitic potential of such a dicaryon is determined by the genotypes of the constituent nuclei. On the basis of this system of interaction of complementary factors for pathogenicity in *U. avenae,* the results of the crosses represented in Table III may be explained on a genetic basis by the analysis of these same crosses presented in Table IV. This analysis indicates why, genetically, the mating of sporidia from different promycelia may result in either strongly-pathogenic or relatively avirulent dicaryons. The weakly-pathogenic dicaryons (Nos. 9, 10, 17 and 18) are represented by aaBb genotypes and as such are characterized by the lack of one dominant factor (A—B—) necessary for high infection.

A careful study of the genotypes and the corresponding compatibility groups of each sporidium in Table IV reveals that a given sporidium is characterized by either a +AB or a —ab genotype. It is postulated, therefore, that the factors +AB are borne on one chromosome and the factors —ab on another. Exceptions to this type of distribution of pathogenicity factors and compatibility type

TABLE IV. AN ANALYSIS OF INTERRACIAL HYBRID (F_1) PROGENY OF USTILAGO AVENAE SHOWING THE GENOTYPIC CONSTITUTION OF PROGENY PRODUCING CONTRASTING HIGH AND LOW INFECTION

Parent Spores and Cross	Sporidium				Dicaryon		
	No.[a]	Group	Genotype	Hybrid Number[b]	Sporidial Combination[c]	Genotype	Infection Level
Cross 1 (A5×A6)	51	+	AB	1	51 × 63	AaBb	high
	52	−	ab	2	51 × 64	AaBb	high
	53	−	ab	3	52 × 61	AaBb	high
	54	+	AB	4	52 × 62	AaBb	high
	61	+	AB	5	53 × 61	AaBb	high
	62	+	AB	6	53 × 62	AaBb	high
	63	−	ab	7	54 × 63	AaBb	high
	64	−	ab	8	54 × 64	AaBb	high
Cross 2 (A5×A6)	51	+	aB	9	51 × 63	aaBb	low
	52	−	ab	10	51 × 64	aaBb	low
	53	−	Ab	11	52 × 61	AaBb	high
	54	+	AB	12	52 × 62	AaBb	high
	61	+	AB	13	53 × 61	AABb	high
	62	+	AB	14	53 × 62	AABb	high
	63	−	ab	15	54 × 63	AaBb	high
	64	−	ab	16	54 × 64	AaBb	high
Cross 3 (A5×A7)	51	+	aB	17	51 × 72	aaBb	low
	52	−	ab	18	51 × 74	aaBb	low
	53	−	Ab	19	52 × 71	AaBb	high
	54	+	AB	20	52 × 73	AaBb	high
	71	+	AB	21	53 × 71	AABb	high
	72	−	ab	22	53 × 73	AABb	high
	73	+	AB	23	54 × 72	AaBb	high
	74	−	ab	24	54 × 74	AaBb	high
Cross 4 (A6×A7)	61	+	AB	25	61 × 72	AaBb	high
	62	+	AB	26	61 × 74	AaBb	high
	63	−	ab	27	62 × 72	AaBb	high
	64	−	ab	28	62 × 74	AaBb	high
	71	+	AB	29	63 × 71	AaBb	high
	72	−	ab	30	63 × 73	AaBb	high
	73	+	AB	31	64 × 71	AaBb	high
	74	−	ab	32	64 × 73	AaBb	high

[a] The race number is inserted before the sporidium number.
[b] Hybrid numbers correspond to the hybrid numbers in Table III.
[c] The sporidial combinations correspond to those in Table III.

are indicated by the +aB and the —Ab sporidia in Table IV. However, such distributions may be accounted for on the basis of genetic crossovers.

Pathogenicity of F_2 progeny obtained by selfing F_1 hybrids

To obtain further information on the number and nature of factors governing pathogenicity in *U. avenae,* spores from F_1 hybrids were selected for selfing to obtain the F_2 or segregating generation. Since crosses 2, 3, and 4 in Table III were conducted simultaneously during one growing season, spores were selected from among the hybrid (F_1) progeny of these crosses for selfing. Only spores representative of uniformly pathogenic hybrids were selected for these studies. A number of spores from the F_1 hybrids representing crosses A5 × A6, A5 × A7, A6 × A7 were selfed by mating compatible monosporidial lines from each spore to produce four F_2 lines. The reactions of these F_2 progeny are indicated as infection percentages in Table V. In most instances the individual selfed lines expressed pathogenic reactions comparable to those of their respective parent races. In other instances (spores 9, 10, 13, and 14) there were hybrid segregates almost completely lacking in pathogenicity. This irregularity in the pathogenic reactions among F_2 progeny is further evidence of the heterozygosity manifested by the F_1 hybrids (Table III).

In a consideration of the pathogenic reactions expressed by selfed lines the ratios of strongly-pathogenic and weakly-pathogenic lines are designated numerically as 4:0, 3:1, and 2:2. The spores characterized by 3:1 and 2:2 pathogenicity ratios among the F_2 lines were derived only from those crosses showing 3:1 ratios for pathogenicity in the F_1. In contrast to this, spores derived from the cross characterized by a 4:0 pathogenicity ratio in the F_1 all resulted in uniformly-infecting or 4:0 ratios among the F_2 hybrids represented by the data in Table V.

In this investigation the interaction of two complementary factors governing pathogenicity is postulated for these reasons: (1) Explanation of the 2:2, 3:1, and 4:0 pathogenicity rations found in Tables III and V is most simply made on a two-factor basis. (2) Crosses 1 and 2 in Table IV have common parents, but segregation of pathogenicity factors and compatibility groups for each A5 parent is different. This difference implies that more than one factor for pathogenicity is present in the same parent race. (3) A two-factor

TABLE V. PATHOGENIC REACTIONS OF F_2 PROGENY OBTAINED FROM F_1 HYBRIDS OF INTERRACIAL CROSSES IN USTILAGO AVENAE

Spore Number and Cross	F_1 Parent and Genotype[a]	Sporidial Matings	Percentage of Infection						Pathogenicity Ratio
			Anthony	Black Diamond	Victory	Gothland	Monarch	Camas	
7-(A5xA6)	F_1 No. 11 (AaBb)	1x2	99	64	63	90	8	0	4:0
		1x4	91	93	78	74	1	0	
		3x2	100	85	76	92	5	0	
		3x4	95	80	84	83	3	0	
8-(A5xA6)	F_1 No. 11 (AaBb)	1x2	93	93	92	93	5	0	4:0
		1x3	86	86	88	81	8	0	
		4x2	98	93	96	99	10	0	
		4x3	90	72	89	78	4	0	
9-(A5xA6)	F_1 No. 11 (AaBb)	1x2	96	66	84	88	3	0	2:2
		1x4	15	7	11	10	1	0	
		3x2	92	97	92	92	2	0	
		3x4	7	3	4	1	0	0	
10-(A5xA6)	F_1 No. 11 (AaBb)	1x2	2	0	12	4	29	0	3:1
		1x3	91	65	94	63	0	0	
		4x2	97	98	92	97	12	0	
		4x3	99	85	96	96	0	0	
11-(A5xA6)	F_1 No.11 (AaBb)	1x3	77	72	73	62	8	0	4:0
		1x4	69	71	76	71	0	0	
		2x3	85	67	62	83	93	0	
		2x4	81	59	63	86	8	0	

(Cont.)

TABLE V—*(Continued)*

Spore Number and Cross	F_1 Parent and Genotype[a]	Sporidial Matings	Percentage of Infection						Pathogenicity Ratio
			Anthony	Black Diamond	Victory	Gothland	Monarch	Camas	
12-(A5xA6)	F_1 No. 14 (AABb)	1x3	43	22	21	67	74	0	4:0
		1x4	72	70	79	54	2	0	
		2x3	81	81	76	79	19	0	
		2x4	100	85	87	91	3	0	
13-(A5xA7)	F_1 No. 21 (AABb)	1x3	93	91	96	96	19	5	3:1
		1x4	98	86	88	95	12	0	
		2x3	15	0	2	9	0	0	
		2x4	98	89	86	96	14	0	
14-(A5xA7)	F_1 No. 21 (AABb)	1x3	98	94	87	94	96	0	3:1
		1x4	96	95	90	98	9	1	
		2x3	93	94	94	95	7	0	
		2x4	9	0	0	1	6	0	
15-(A5xA7)	F_1 No. 24 (AaBb)	1x2	84	83	94	92	0	0	4:0
		1x3	95	84	94	92	9	0	
		4x2	93	91	94	92	8	0	
		4x3	91	78	91	97	98	0	
16-(A5xA7)	F_1 No. 24 (AaBb)	1x2	94	81	91	86	11	0	4:0
		1x4	89	79	92	90	93	81	
		3x2	97	78	95	89	0	0	
		3x4	95	86	90	95	7	0	
17-(A5xA7)	F_1 No. 24 (AaBb)	1x2	89	89	90	94	0	93	4:0
		1x4	88	86	89	95	9	0	
		3x2	96	84	98	97	12	0	
		3x4	93	87	90	95	99	0	

(Cont.)

TABLE V—*(Continued)*

Spore Number and Cross	F_1 Parent and Genotype[a]	Sporidial Matings	Percentage of Infection						Pathogenicity Ratio
			Anthony	Black Diamond	Victory	Gothland	Monarch	Camas	
18-(A5xA7)	F_1 No. 24 (AaBb)	1x3	100	100	100	100	26	100	4:0
		1x4	100	82	86	46	0	0	
		2x3	100	100	100	100	100	3	
		2x4	98	100	96	97	5	2	
19-(A6xA7)	F_1 No. 31 (AaBb)	1x2	92	80	84	99	99	2	4:0
		1x4	97	89	91	91	97	0	
		3x2	92	88	88	88	97	74	
		3x4	98	90	79	89	88	4	
20-(A6xA7)	F_1 No. 31 (AaBb)	1x2	79	74	67	85	86	0	4:0
		1x4	67	67	56	66	76	0	
		3x2	73	64	59	74	80	0	
		3x4	68	74	72	78	85	38	
21-(A6xA7)	F_1 No. 31 (AaBb)	1x2	91	85	72	83	67	0	4:0
		1x3	95	76	73	74	85	0	
		4x2	97	69	81	98	100	0	
		4x3	99	53	79	76	93	72	
22-(A6xA7)	F_1 No. 31 (AaBb)	1x2	26	37	31	47	42	0	4:0
		1x3	39	44	55	56	78	26	
		4x2	70	52	72	83	76	0	
		4x3	77	85	69	78	86	0	
A5		.....[b]	88	69	80	82	0	0	.....
A6		.....	75	59	53	66	75	0	
A7		.....	78	69	61	80	91	53	

[a] F_1 hybrid number and genotype correspond to those in Table IV indicating the source and genetic constitution of spores selected for selfing.

[b] Spore inoculum of parent races was used in growing the controls.

system is necessary to explain the genotypes of the hybrid (F_1) spores in hybrid lines 11 and 21 (Table III), which on selfing gave rise simultaneously to the progeny in Table V characterized by 2:2 and 3:1 pathogenicity ratios. A single gene system is inadequate to explain the origin of these F_2 ratios from specific F_1 genotypes, as indicated in Table IV.

In relating the data in these experiments with those obtained for the same pathogen by Holton (22, 26) and Nicolaisen (38), many similarities in pathogenicity ratios are evident. Holton's data indicate manifestations of 4:0, 3:1, and 2:2 pathogenicity ratios among selfed and outcrossed progenies of *U. avenae*. Similarly, data published by Nicolaisen (38) pertaining to selfed spores indicate the expression of 4:0, 3:1, and 2:2 pathogenicity ratios among the constituent lines. Nicolaisen's data represent seventeen 4:0, four 3:1, and one 2:2 ratios. However, data from an equal number of selfed spores in Tables II and V in the present study indicate eighteen 4:0, three 3:1, and one 2:2 pathogenicity ratios. Thus, remarkable agreement exists between these three independent sources of data. This fact serves to emphasize the need for a reliable genetic analysis of factors governing the pathogenic reactions of races of *U. avenae*.

Genetic analysis of F_2 progeny from F_1 hybrids

The pattern of mated sporidia isolated from a germinated spore of *U. avenae* depends on the distribution of compatibility groups. These groups are designated as plus (+) and minus (−) to indicate opposite mating types among sporidia. The consistent production of only four compatible combinations per spore indicates the formation of equal numbers of plus and minus sporidia on each promycelium. From the data presented in Tables II and V it is apparent that the mating of sporidia from a given promycelium always conforms to one of three basic patterns or their reciprocals as follows:

(1) 1×2, 1×3, 4×2, 4×3; (+−−+) or (−++−)
(2) 1×2, 1×4, 3×2, 3×4; (+−+−) or (−+−+)
(3) 1×3, 1×4, 2×3, 2×4; (++−−) or (−−++)

These numerical patterns of sporidial matings are governed by the underlying arrangements of the compatibility groups as indicated in parenthesis. They are accounted for by the time of reduction division for plus and minus compatibility groups in the promycelium. If segregation for compatibility occurred in the first nuclear division

pattern 3 would result, whereas if segregation for compatibility occurred in the second division, patterns 1 or 2 would result. Of the twenty-two spores represented in Tables II and V, six are characterized by pattern 1, nine by pattern 2, and seven by pattern 3. For comparison, Nicolaisen's (38) data representing twenty-two selfed spores indicate that five are characterized by pattern 1, six by pattern 2, and eleven by pattern 3. Thus, among these forty-four spores of *U. avenae,* segregation for compatibility occurred in the first nuclear division in eighteen instances and in the second nuclear division twenty-six times.

In a genetic analysis of parents and hybrids the distribution of factors for pathogenicity in each plus and minus sporidium is of primary importance. The data indicate, as was pointed out, that possibly a linkage exists between factors governing pathogenicity and factors determining compatibility. Since the pathogenic reaction of each selfed line is governed by the genetic constitution of the dicaryon involved, the reactions of all lines may be accounted for on a genetic basis. The three basic patterns of sporidial matings among the haploid progeny from a given spore are presented in Table VI.

TABLE VI. THREE BASIC PATTERNS OF SEGREGATION FOR COMPATIBILITY AMONG SELFED PARENTS AND HYBRIDS OF USTILAGO AVENAE

		Basic Pattern Types		
Pattern Number	Sporidium Number[a]	Compatibility Group[b]	Sporidial Combinations	Parent and Hybrid Spores[c]
1	1	+	1×2	1,8
	2	−	1×3	15,21
	3	−	4×2	22
	4	+	4×3	
2	1	+	1×2	2,3,5
	2	−	1×4	7,16
	3	+	3×2	17,19
	4	−	3×4	20
3	1	+	1×3	4,6,11
	2	+	1×4	12
	3	−	2×3	18
	4	−	2×4	

[a] Sporidia are numbered as 1 to 4 from the apex of the promycelium to the base.

[b] Plus (+) and minus (−) signs indicate sporidia of opposite compatibilty groups. Sporidium No. 1 is always arbitrarily designated as plus (+).

[c] Spore numbers correspond to those in Tables II and V.

The existence of certain F_2 lines almost completely lacking in pathogenicity is evident from the data in Table V. These weakly-pathogenic lines result in 2:2 and 3:1 pathogenicity ratios among the progeny from a hybrid spore. Undoubtedly the relative lack of virulence in these lines is based on the genetic constitution of the dicaryon involved. The genetic analyses of the selfed spores characterized by 2:2 and 3:1 pathogenicity ratios are presented in Table VII. In these analyses it is postulated that the low infections ex-

TABLE VII. GENETIC ANALYSIS OF HYBRID SEGREGATES IN USTILAGO AVENAE CHARACTERIZED BY 2:2 AND 3:1 PATHOGENICITY RATIOS

	Sporidium			Dicaryon		
Spore Number and Ratio[a]	Number	Group	Genotype	Combination	Genotype	Infection level
9-AaBb (2:2)	1	+	Ab	1×2	AABb	high
	2	−	AB	1×4	Aabb	low
	3	+	aB	3×2	AaBB	high
	4	−	ab	3×4	aaBb	low
10-AaBb (3:1)	1	+	ab	1×2	aabb	low
	2	−	ab	1×3	AaBb	high
	3	−	AB	4×2	AaBb	high
	4	+	AB	4×3	AABB	high
13-AABb (3:1)	1	+	AB	1×3	AABb	high
	2	+	Ab	1×4	AABB	high
	3	−	Ab	2×3	AAbb	low
	4	−	AB	2×4	AABb	high
14-AABb (3:1)	1	+	AB	1×3	AABB	high
	2	+	Ab	1×4	AABb	high
	3	−	AB	2×3	AABb	high
	4	−	Ab	2×4	AAbb	low

[a] Spore numbers and pathogenicity ratios correspond to those in Table V.

pressed by certain lines are due to a lack of either one or both dominant factors governing pathogenicity. The genotypic constitutions (AAbb, Aabb, aaBb, or aabb) of the avirulent dicaryons undoubtedly account for their weak infection potential as indicated by the analyses in Table VII.

Pathogenic reactions and genetic analysis of selfed hybrid (F_2) lines on Monarch oats

The original objective of this study was to ascertain the genetic basis for the differences in pathogenicity between races A5, A6, and

A7 of *U. avenae*. One of the questions raised was: Why, genetically, does one race (A5) infect only four varieties while another race (A6) infects these same four varieties and one additional variety (Monarch) as well? Since races A5 and A6 are separable by distinct pathogenic reactions on Monarch (Tables I and II) it is evident that these clearcut differences in pathogenicity are governed by heritable factors. From the foregoing discussion it is apparent that all three races possess a common genetic system governing pathogenicity on the four susceptible varieties—Anthony, Black Diamond, Victory, and Gothland. The specific objective now is to determine the number and nature of factors present in A6, in addition to those present in A5, which account for the wider or extended pathogenicity of A6 on Monarch oats. Since Monarch is used to separate races A5 and A6, it also serves as a key differential variety for studying the pathogenic reactions of the hybrid (F_1) lines as well as the segregating (F_2) progeny of these races. A panicle of Monarch oats showing typical symptoms of infection by *U. avenae* is shown in Figure 7.

The genetic basis for the additional pathogenic potential of race A6 on Monarch in contrast to that of race A5 is indicated in the pathogenicity of the hybrid F_1 and F_2 lines. An analysis of pathogenicity on Monarch involves consideration also of both A5 $\times$ A6 and A5 $\times$ A7 crosses, since A7 is also pathogenic on this differential variety. The pathogenicity of the F_1 hybrids representing these two crosses is recorded in Table III. All the F_1 lines (Nos. 9 to 24 inclusive) are characterized by relatively low pathogenic reactions on Monarch. The pathogenicity of all sixteen hybrid (F_1) lines on Monarch is within the range of 1 to 9 per cent infection. Similar reactions on Monarch are also apparent among the hybrid (F_1) lines of cross 1 in Table III. From these data it is evident that the races characterized by the wider range of pathogenicity on Monarch are only weakly manifested in the F_1 on this differential variety.

It is postulated that a single gene "M" or its recessive allele "m" governs the pathogenic reactions of these races and their hybrids on Monarch. Thus A5 is probably characterized by the homozygous recessive constitution "mm," while A6 and A7 both possess the homozygous dominant "MM" genotype. The consistently low infections by the F_1 hybrids on this variety may, therefore, be attributed to their heterozygous genotypes "Mm" for this locus, assuming

that dominance is lacking. The evidence for a single-gene control of infection on Monarch is further substantiated by the data presented in Table VIII. These data represent a genetic analysis of the pathogenic reactions of the segregating hybrid (F_2) progeny on Monarch oats.

This table reveals that the 4:0 pathogenicity ratios on Monarch may be explained by a heterozygous genotype basis (Mm:Mm:Mm:-Mm) whereas a 3:1 ratio may be accounted for by both homozygous and heterozygous (MM: 2Mm: mm) distribution of pathogenicity factors. It is postulated that the homozygous dominant genotype (MM) results in the high infections (74 to 100 per cent) by the hybrid (F_2) lines characterized by 3:1 pathogenicity ratios on Monarch. These ratios occurred in the frequency of five 4:0 and seven 3:1 respectively. It is concluded, therefore, that the additional pathogenicity of races A6 and A7 on Monarch is contingent on the expression of one additional factor pair (MM) governing pathogenicity on this differential variety.

Pathogenic reactions and genetic analysis of hybrid (F_2) lines on Camas oats

A consideration of the nature and number of heritable factors governing the consistent differences in pathogenicity between races of *U. avenae* in this study includes a genetic analysis of race A7. Among the twenty known races of the loose-smut pathogen, this race is unique. It is the only known race of *U. avenae* which expresses itself by a non-sporulating type of infection on the differential variety Camas. In contrast to this, race A2, another Camas race (Fig. 1), produces a typically loose or powdery sorus. It is apparent that the ability of A7 to produce this non-sporulating infection probably is due to insufficient host-parasite compatibility to allow sporulation. The infection of Camas by A7 is further characterized by a pronounced dwarfing of infected plants.

In evaluating the genetic differences between the races in this study another question was raised: Why does one race (A6) infect only five varieties, while another race (A7) infects these same five varieties and one additional variety (Camas) as well? The procedure used to elucidate the genetic differences between these races was similar to that followed in analyzing the pathogenic reactions of other hybrids on Monarch.

A study was made of the hybrid (F_1) lines representing crosses

TABLE VIII. Pathogenic Reactions as Shown by Infection Percentages and Genetic Analysis of Hybrid F_2 Lines of Ustilago avenae on Monarch Oats

	Spore Numbers[a]											
	Cross of A5 x A6						Cross of A5 x A7					
	7	8	9	10	11	12	13	14	15	16	17	18
	8	5	3	*29*	8	*74*	19	*96*	0	11	0	26
Percentage of Infection	1	8	1	0	0	2	12	9	9	*93*	9	0
on Monarch by Selfed	5	10	2	12	*93*	19	0	7	8	0	12	*100*
Hybrid (F_2) Progeny	3	4	0	0	8	3	14	6	*98*	7	*99*	5
	Mm	Mm	Mm	Mm	Mm	*MM*	Mm	*MM*	mm	Mm	mm	Mm
Genetic Constitution of	Mm	Mm	Mm	Mm	mm	Mm	Mm	Mm	Mm	*MM*	Mm	mm
Above F_2 Dicaryons	Mm	Mm	Mm	Mm	*MM*	Mm	Mm	Mm	Mm	mm	Mm	*MM*
	Mm	Mm	Mm	Mm	Mm	mm	Mm	mm	*MM*	Mm	*MM*	Mm
Pathogenicity Ratio[b]	4:0	4:0	4:0	4:0	3:1	3:1	4:0	3:1	3:1	3:1	3:1	3:1

[a] Spore numbers correspond to those in Table V.
[b] Ratio of Monarch Resistant to Monarch Susceptible hybrids.

3 and 4 in Table III, in which A7 is one of the parent races. The pathogenicity of the hybrid lines on Camas was either extremely low or non-existent. Six of the sixteen lines representing these two crosses were each characterized by trace (1 to 2 per cent) infections while the remaining ten lines showed zero infections on Camas. In one cross of A6 × A7 the F_1 hybrids all failed completely to infect Camas, suggesting dominance of the weaker parent race (A6) reaction. Table IX was prepared to determine the genetic basis for these pathogenic reactions on Camas, and to facilitate a comparative study of them.

It is apparent from the data presented in Table IX that a single factor pair governs the pathogenic reactions of the parent races and their hybrids on the variety Camas. A comparison of Table VIII with Table IX indicates that the hybrid progeny of a given spore (No. 14 and 15) may result in a 3:1 pathogenicity ratio on Monarch and simultaneously impart a 4:0 ratio on Camas. Further evidence of a distinct factor controlling Camas infection is found among the selfed progeny of Spore 17 in Table V, where the dominant genes governing pathogenicity for these two varieties are apparently carried in separate hybrid dicaryons.

It is postulated that a gene "C" and its recessive allele "c" govern the pathogenic reactions of races A5, A6, and A7 on Camas oats. Races A5 and A6 are both characterized by the homozygous recessive "cc" constitution whereas A7 is apparently pathogenic by virtue of its homozygous dominant "CC" genotypic constitution. Weak or trace infections by the hybrid (F_1) lines on Camas may be attributed to the heterozygous constitution "Cc," assuming that dominance is lacking. The F_2 ratios for pathogenicity on Camas probably are due to the presence of Cc:Cc:Cc:Cc and CC : 2Cc : cc genotypes of each set of four hybrid F_2 lines characterized by 4:0 and 3:1 ratios for infection on Camas, respectively.

Pathogenic reactions of hybrid (F_3) lines and their genetic significance

The three races of *U. avenae* used in these studies are readily differentiated by their consistent and distinct pathogenic reactions on the varieties Monarch and Camas. For this reason these two varieties are indispensable in evaluating the pathogenic reactions of the hybrid (F_1) lines as well as in ascertaining the segregating ratios expressed by the hybrid F_2 lines. Because of this apparent utility

TABLE IX. PATHOGENIC REACTIONS AS SHOWN BY INFECTION PERCENTAGES AND GENETIC ANALYSIS OF HYBRID F_2 LINES OF USTILAGO AVENAE ON CAMAS OATS

	Spore Number[a]									
	Cross of A5 x A7						Cross of A6 x A7			
	13	14	15	16	17	18	19	20	21	22
	5	0	0	0	*93*	*100*	2	0	0	0
Percentage of Infection	0	1	0	*81*	0	0	0	0	0	*26*
on Camas by Selfed	0	0	0	0	0	3	*74*	0	0	0
Hybrid (F_2) Progeny	0	0	0	0	0	2	4	*38*	*72*	0
	Cc	Cc	Cc	Cc	*CC*	*CC*	Cc	cc	cc	Cc
Genetic Constitution of	Cc	Cc	Cc	*CC*	Cc	Cc	cc	Cc	Cc	*CC*
Above F_2 Dicaryons	Cc	Cc	Cc	cc	Cc	Cc	*CC*	Cc	Cc	cc
	Cc	Cc	Cc	Cc	cc	cc	Cc	*CC*	*CC*	Cc
Pathogenicity Ratio[b]	4:0	4:0	4:0	3:1	3:1	3:1	3:1	3:1	3:1	3:1

[a] Spore numbers correspond to those in Table V.
[b] Ratio of Camas Resistant to Camas Susceptible.

these two varieties have been called the "key differentials" in this study.

In the foregoing sections the pathogenic reactions of the parent races, their F_1 hybrids, and their segregating F_2 progeny have been evaluated. Separate discussions were devoted to a specific consideration of the genetic basis for the differences in pathogenicity of the parent races on Monarch and Camas. The data in Tables VIII and IX indicated 4:0 and 3:1 pathogenicity ratios characterizing the F_2 progeny on each of these varieties. These pathogenic reactions were explained on a single factor basis for each variety respectively. The principal value of growing an F_3 progeny is to obtain supporting evidence for the genetic analysis proposed for the F_2 segregates. This confirmation is possible because the homozygotes in a population maintain their genetic identity whereas the heterozygotes segregate in the usual 1:2:1 manner. This behavior of the heterozygotes accounts for the trace or intermediate levels of infections on the key differentials in the F_3 where no infection was present in the F_2.

The F_3 data in Table X represent the pathogenic reactions of segregate lines derived from hybrid F_2 spores representing each of three race crosses. These data are presented primarily as support-

TABLE X. PATHOGENIC REACTIONS OF F_3 SEGREGATE LINES FROM INTERRACIAL HYBRID F_2 SPORES OF USTILAGO AVENAE

Spore Number[a] and Cross	Sporidial Combination	Percentage of Infection					
		Anthony	Black Diamond	Victory	Gothland	Monarch	Camas
7-(A5×A6)	1×2	68	14	26	30	12	0
	1×4	86	32	65	75	19	0
	3×2	56	23	40	47	21	0
	3×4	85	27	47	69	22	0
11-(A5×A6)	1×3	79	46	54	55	29	0
	1×4	75	38	59	76	0	6
	2×3	74	39	31	25	80	18
	2×4	80	40	58	65	31	0
12-(A5×A6)	1×3	74	46	60	73	91	0
	1×4	78	36	59	65	26	0
	2×3	70	41	62	72	25	0
	2×4	82	32	64	61	0	0

(Cont.)

TABLE X (*Continued*)

Spore Number[a] and Cross	Sporidial Combination	Percentage of Infection					
		Anthony	Black Diamond	Victory	Gothland	Monarch	Camas
15-(A5×A7)	1×2	72	36	61	64	0	4
	1×3	69	45	64	75	42	23
	4×2	71	40	43	63	48	21
	4×3	73	45	36	62	79	39
16-(A5×A7)	1×2	73	36	50	64	19	13
	1×4	78	37	52	79	90	68
	3×2	74	56	69	74	0	0
	3×4	65	38	58	68	32	11
17-(A5×A7)	1×2	69	43	52	70	1	58
	1×4	75	52	64	81	26	13
	3×2	82	47	55	75	31	17
	3×4	77	51	52	77	89	2
19-(A6×A7)	1×2	75	58	53	70	83	5
	1×4	74	70	49	74	85	0
	3×2	55	72	32	52	73	48
	3×4	74	58	50	71	93	8
20-(A6×A7)	1×2	62	40	43	76	85	0
	1×4	71	51	37	67	84	5
	3×2	51	34	25	49	72	5
	3×4	64	58	22	63	76	42
21-(A6×A7)	1×2	85	66	51	72	87	0
	1×3	74	39	32	71	68	8
	4×2	83	56	15	62	80	8
	4×3	82	54	41	81	79	78
22-(A6×A7)	1×2	81	53	44	69	64	4
	1×3	69	32	41	64	79	45
	4×2	62	32	37	54	76	0
	4×3	54	26	23	40	76	3
A5	...[b]	88	69	80	82	0	0
A6	...	75	59	53	66	75	0
A7	...	78	69	61	80	91	53

[a] Spore numbers correspond to those in Tables V, VIII, and IX.
[b] Spore inoculum of parent races was used in growing the controls.

ing evidence for the existence of separate genes for Monarch and Camas infection, respectively. They indicate 4:0 and 1:2:1 ratios for pathogenicity on the differential variety Monarch. A direct comparison of the parental (F_2) reactions in Table VIII with the

resulting F_3 reactions indicates a predictable genetic behavior pertaining to pathogenicity on Monarch. All the spores (Nos. 11, 12, 15, 16, and 17) characterized by a 3:1 pathogenicity ratio on Monarch in the F_2 resulted in the predicted 1:2:1 ration on Monarch in the F_3. Similarily, a 4:0 ratio on Monarch characterizing spore 7 in the F_2 (see Table VIII) resulted in a 4:0 ratio on this variety in the F_3 (see Table X) as was expected.

A careful comparison of the pathogenic reactions of the F_2 lines in Table IX on the variety Camas with that of the resulting F_3 derivatives in Table X also indicates a consistency in genetic behavior. Thus, every spore (Nos. 16, 17, 19, 20, 21, and 22) characterized by a 3:1 pathogenicity ratio on Camas in the F_2 resulted in the expected 1:2:1 ratio on Camas in the F_3. A minor exception, however, is spore 15 which is characterized by a 4:0 pathogenicity ratio in the F_2 but segregated irregularly for pathogenicity on Camas in the F_3 (see Table X).

The individual 1:2:1 pathogenicity ratios expressed by the F_3 segregates (Table X) on Monarch and Camas are directly related to the 3:1 ratios characterizing the corresponding F_2 parents in Tables VIII and IX. These 1:2:1 pathogenicity ratios characterizing the F_3 segregates confirm the genotypic constitutions postulated for the F_2 dicaryons with respect to the Monarch and Camas factors for pathogenicity in Tables VIII and IX, respectively.

Origin of a new pathogenic strain of Ustilago avenae through hybridization

The heritable nature of factors governing pathogenicity in the smut fungi is well-established. It also has been shown that from crosses between species and races characterized by well-defined contrasting pathogenicity, segregates may occur which differ in pathogenicity from both parents. In the oat smuts, for example, Holton (20) obtained a synthetic strain of *U. kolleri* from the hybrid *U. avenae* × *U. kolleri* which was more widely pathogenic than either parent, and likewise, a strain of the buff smut was obtained which differed pathogenically from both its parents.

In this study of the inheritance of pathogenicity factors in *Ustilago avenae,* a new pathogenic strain of the fungus was detected among the hybrid F_2 derivatives of spore 17 (see Table V). Apparently this new hybrid originated through segregation and recombination of factors governing pathogenicity as indicated by the data presented in Table XI.

TABLE XI. PATHOGENIC REACTIONS OF PARENTS AND HYBRIDS PERTAINING TO THE ORIGIN OF A NEW STRAIN OF USTILAGO AVENAE

Race or Hybrid Identity	Inoculum Type	Reference	Percentage of Infection									
			Anthony	Black—Diamond	Victory	Gothland	Monarch	Fulghum	Black—Mesdag	Camas	Nicol	Lelina
A5 Parent	Spores	Table I	88	69	80	82	0	....	....	0	....	....
A7 Parent	Spores	Table I	78	69	61	80	91	....	....	53	....	....
F_1 Hybrid	54×74	Table III	99	93	86	95	5	....	....	2	....	....
F_2 Hybrid (Segregates)	1×2[a]	Table V (Spore 17)	89	89	90	94	*0*	....	....	93	....	....
	1×4		88	86	89	95	9	....	....	0	....	....
	3×2		96	84	98	97	12	....	....	0	....	....
	3×4		93	87	90	95	99	....	....	0	....	....
F_3 Hybrid	Spores	...[b]	97	93	94	97	*3*	5	2	96	6	0
F_4 Hybrid	Spores	...[b]	84	78	61	73	*0*	3	2	85	12	0
New Strain	.....	Fig. 1	S	S	S	S	*R*	R	R	S	R	R

[a] Combination 1×2 imparted a pathogenic reaction that does not resemble that of any known race in Figure 1.

[b] The F_3 and F_4 pathogenic reactions on Monarch are comparable to that of their F_2 parent.

The existence of separate factors governing the pathogenic reactions of loose-smut dicaryons on Monarch and Camas, respectively, has been discussed. A specific consideration of the existence of a new pathogenic entity on the basis of the data presented in Table XI is further evidence of the existence of distinct factors governing pathogenicity on these two varieties. A careful scrutiny of the sporidial combinations of the selfed lines of spore 17 in this table reveals that the MM and CC factors are located in separate hybrid dicaryons. Since the factors governing these reactions are apparently separable, it is assumed that they are discrete or distinct heritable units.

The pathogenicity of the new strain of Camas loose smut is clearly different from that of either parent race (A5 or A7) and also from that of the hybrid F_1 line shown in Table XI. A further comparison of this reaction with the original race-reaction chart (Fig. 1) for the pathogen indicates that it is unique among the known races of *U. avenae*. This strain resembles A7 in the nonsporulating type of infection on Camas but differs from it patho-

genically by its relative lack of infection on the variety Monarch. Furthermore, it differs from race A2 by its pathogenicity on Gothland (Fig. 1) and also by the sorus type on Camas, since A2 produces a sporulating type of sorus on this variety. The pathogenicity of this new Camas strain of loose smut persisted through the F_3 and the F_4 generations (Table XI), and it apparently represents an additional race of *U. avenae* characterized by its unique pathogenic reaction on the standard differential varieties of oats.

Discussion and Conclusions

In this study of the inheritance of factors governing pathogenicity in *U. avenae,* emphasis was placed on an analysis of the pathogenic reactions of hybrid F_1 progeny and their F_2 derivatives from crosses of races A5, A6, and A7. The appearance in the F_1 of these hybrids of certain lines characterized by a uniform and low level of pathogenicity on all the differential varieties constituted an unexpected development of paramount importance to these investigations. The reappearance of these virtually innocuous lines among the selfed F_2 progeny served to confirm the genetic nature of this phenotypic effect. Apparently the contrastingly weak pathogenicity expressed by these avirulent dicaryons resulted from heterozygous factors governing pathogenicity. A study of these pathogenically aberrant hybrids revealed that the heterozygous condition of the parent races undoubtedly contributed toward these irregularities in pathogenicity. A diagrammatic scheme which serves to recapitulate briefly the origin and frequency of pathogenicity ratios which indicate heterozygosity is shown in Figure 8.

An important feature of the expression manifested by the weakly-pathogenic lines in the hybrid populations is the consistent uniformity of such low infections on all the differential varieties. A consideration of the genetics of pathogenicity within the scope of this study included an analysis of this weak pathogenicity. On the basis of the experimental results obtained, the investigation resolved itself into three categories, as follows:

(1) An analysis of the genetic factors governing the uniformly-strong or uniformly-weak expression of pathogenicity on the four susceptible varieties: Anthony, Black Diamond, Victory and Gothland.

(2) A genetic analysis of the pathogenic reactions of the parent races and their hybrid F_1, F_2, and F_3 progeny on Monarch oats.

(3) A genetic analysis of the pathogenic reactions of the parent races and their hybrid F_1, F_2 and F_3 progeny on Camas oats.

The experimental data show that the factors governing pathogenicity on the four susceptible varieties behaved as heritable units. The fact that a given line expressed either pronounced infection or contrastingly-weak infection on all varieties indicates genetic control of pathogenicity. For this reason, an interaction of factors was postulated to explain the uniformly high or low pathogenic reactions by hybrid dicaryons on the four susceptible varieties. The data further indicate that this expression of pathogenicity is not merely a summation of the separate effects produced by two genes alone but rather that it represents a pronounced effect imparted by their interaction.

In addition to the above mechanism of the interaction of complementary factors governing pathogenicity in *U. avenae,* the existence of discrete factors governing the pathogenic reactions on the key differential varieties, Monarch and Camas respectively, has been substantiated with experimental evidence. It is apparent that a specific dicaryon may be pathogenic on Monarch but not on Camas, or it may be pathogenic on both varieties. However, the detection of a hybrid dicaryon which is highly pathogenic on Camas but not on Monarch strengthens the concept that separate pathogenicity factors govern the pathogenic reactions on these varieties. It furthermore marks the origin, through induced hybridization, of a new pathogenic strain of *U. avenae.* Holton (29) pointed out that, in practical experience with this fungus, new races are encountered frequently, suggesting sufficient intermingling of distinct types to create a dynamic potential with respect to pathogenicity. The experimental production of a new Camas strain of the loose-smut pathogen in these studies further supports this observation.

These experimental results leave no doubt as to the heritable nature of pathogenicity factors in *U. avenae,* and the nature and number of discrete factors that govern the pathogenic reactions of races A5, A6, and A7 were postulated on this basis. The genetic factors detected in these specific races within the scope of this study are presented in summarized form in Table XII. This contribution pertaining to the inheritance of factors governing pathogenicity in this species is sufficiently meaningful to warrant further investigation into the basic aspects of the problem. Since the smut fungi

TABLE XII. SUMMARY OF THE GENETIC FACTORS GOVERNING THE PATHOGENIC REACTIONS OF RACES A5, A6, A7 AND A NEW STRAIN OF UTILAGO AVENAE

Race	Reference	Differential Varieties and Factor Symbols		
		A—BD—V—G*	Monarch	Camas
A5	Tables I, II	A — B —	m m	c c
A6	Tables I, II	A — B —	*M M*	c c
A7	Tables I, II	A — B —	M M	C C
New Strain	Table XI	A — B —	*m m*	C C

* Letters "A—BD—V—G" refer to the differential varieties of oats as follows: Anthony, Black Diamond, Victory and Gothland, respectively.

are not unique among plant-pathogenic fungi with respect to variation resulting from hybridization, further elucidation of the genetic basis of pathogenicity in the smuts may have far-reaching implications in the science of plant pathology.

SUMMARY

1. Three races of *Ustilago avenae,* characterized by closely-related but well-defined pathogenic reactions, were selected for a genetic study of pathogenicity. The results of selfing experiments indicated that these parent races possessed a high degree of homozygosity for pathogenicity although heterozygosity was not precluded.

2. The pathogenic reactions of the interracial hybrid F_1 progeny were characterized by either 4:0 or 3:1 ratios of strongly-pathogenic to weakly-pathogenic hybrids. The occurrence of 4:0 F_1 ratios indicated homozygosity of factors governing pathogenicity in the parents whereas the 3:1 ratios indicated heterozygosity.

3. Selfing the F_1 gametic progeny produced F_2 hybrids which were characterized by 4:0, 3:1 and 2:2 ratios of strongly-pathogenic to weakly-pathogenic lines. The F_1 hybrids that were characterized by 3:1 ratios gave rise to F_2 progeny with 4:0, 3:1 and 2:2 ratios, whereas 4:0 F_1 hybrids yielded F_2 progeny with 4:0 ratios only.

4. A study of segregating F_3 progeny derived from F_2 hybrid spores confirmed the postulated genotypic constitutions assigned to the F_2 dicaryons. These F_3 progeny resulting in 4:0 and 1:2:1

pathogenicity ratios were derived from F_2 parents characterized by 4:0 and 3:1 ratios respectively.

5. The pathogenic reactions of the selfed parent races, the interracial hybrid F_1 progeny, and the hybrid F_2 derivatives were explained on a complementary factor basis. All strongly-pathogenic dicaryons were characterized by AABB, AABb, AaBB, or AaBb genotypes whereas the weakly-pathogenic dicaryons were characterized by AAbb, Aabb, aaBB, aaBb, or aabb genotypes. This postulated analysis accounted for all of the 4:0, 3:1 and 2:2 ratios of strongly-pathogenic to weakly-pathogenic hybrids.

6. The genetic basis for the difference in pathogenicity between races A5 and A6 on the variety Monarch was ascertained. Apparently one factor (MM, Mm, or mm) governed the pathogenic reactions on Monarch. Race A5 was characterized by an "mm" genotype, whereas race A6 possessed an "MM" genetic constitution for this locus.

7. The difference in pathogenicity between races A6 and A7 on the variety Camas is governed by a single factor (CC, Cc, or cc). Race A6 was thus characterized by a "cc" genotype, whereas the infection of Camas by A7 was explained by a "CC" genetic constitution for this locus.

8. The existence of non-allelic factors governing pathogenic reactions on Monarch and Camas respectively was confirmed by the separation of these loci. The experimental production of a hybrid dicaryon characterized by a pronounced lack of infection on Monarch and very high infection on Camas was evidence of the separation of these loci. This hybrid strain of *U. avenae* is considered to be a new pathogenic race of the fungus, having maintained its characteristic pathogenic identity for three successive generations.

9. A genetic analysis of the pathogenicity of the three parent races and their resultant hybrid F_1, F_2, and F_3 progeny indicated that the pathogenic reaction of each dicaryon is contingent on its specific genetic constitution. Thus the genotypye of race A5 was postulated as A—B— mm cc; race A6 as A—B— MM cc; race A7 as A—B— MM CC; and the new hybrid strain as A—B— mm CC with respect to the six differential varieties used in this study.

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A POSSIBLE EXPLANATION FOR THE CYCLIC OCCURRENCE OF DWARF BUNT OF WINTER WHEAT IN THE PACIFIC NORTHWEST*

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Introduction

One of the more perplexing phenomena concerning dwarf bunt on winter wheat in the Pacific Northwest is its spasmodic epidemiology, which tends toward an alternate-year cycle. This cycle has been especially conspicuous in localized areas of relatively new infestations. In Montana, for example, heavy incidence of dwarf bunt was first reported in 1935 (10), and it occurred fairly consistently in alternating years of high and low incidence for about a decade (4). This tendency, together with an occasional inconsistency, has been observed in other localities of the Pacific Northwest and reported informally[1] over the past 20 years. No satisfactory explanation has yet been proposed for these alternating sequences and the occasional breaks in them. The purpose of this paper is to propound a theoretical explanation, based on an accumulation of information on spore germination and certain infection phenomena. The basic consideration with respect to this theory is the effect of light and other factors on spore germination in relation to farming practices.

Spore Germination

A limiting factor in the investigation of dwarf bunt is the slow rate of spore germination in the laboratory. Lowther (6) obtained spore germination in high percentages on water agar after 2 to 3 weeks' incubation at 5° C. This low temperature relationship was later confirmed by several workers (1, 2, 3, 8, 9), some of whom demonstrated that light also stimulates germination of these spores.

The stimulating effect of light was the basis for the theory ad-

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[1] In Annual Reports to the Cereal Crops Section, Field Crops Research Branch, Agricultural Research Service, Plant Industry Station, Beltsville, Maryland.

vanced by Gassner (2) that spore germination and infection in dwarf bunt occurs at or near the soil surface. This theory is supported by recent reports (5, 7) on depth of seeding in relation to dwarf bunt incidence and on spore germination on the soil surface. The infection data reported for 4 depths of seeding, ranging from the soil surface to 4 inches, showed the highest percentage infection in the surface-seeded plots. The percentages of infection decreased as the depth of seeding was increased. In other tests spores germinated on the soil surface after one week of incubation. The significance of these results in relation to the cyclic occurrence of dwarf bunt is revealed in the farming practices of the Pacific Northwest.

Farming Practices

A basic farming practice widely used in wheat production in the Pacific Northwest is the alternate wheat—summer fallow rotation. This system predominates in areas of heavy dwarf bunt infestation, although it has been modified somewhat in recent years in some localities. For example, in the Palouse country of southeastern Washington and adjacent Idaho recropping with peas or wheat sometimes takes the place of summer fallow in the rotation. Still other modifications of the wheat—summer fallow system may be employed in this and other localities of the region. In any case soil management, as determined by the various rotations, probably is directly related to the cyclic nature of dwarf bunt occurrences. This is particularly true as it pertains to the utilization of the wheat stubble. In this connection it is significant that, in harvesting wheat, the cutter bar passes over most of the dwarf-bunt-infected plants, leaving a heavy concentration of smutted heads with the stubble.

The utilization of wheat stubble is an essential requirement of good soil conservation practices in the Pacific Northwest. It may be plowed under to a depth of 6 inches or more, or it may be disked into the surface layer of soil to form a stubble mulch. This latter system is followed mainly in areas where there is need to combat wind erosion. In either case, the severity of dwarf bunt in subsequent wheat crops may be determined by the depth of the stubble and surface soil from the previous crop. How this may operate to produce alternate-year cycles and spasmodic occurrences of dwarf bunt is shown below.

Dwarf Bunt Cycles

Case histories with respect to dwarf bunt occurrences on individual fields are not available. Theoretically, however, on an infested farm where a wheat—summer fallow rotation is followed, a high incidence of the disease may be expected to occur either in alternate crop years or every crop year, depending on whether the stubble from the previous crop is plowed under or worked into a stubble mulch. For example, if the recently harvested crop had a heavy infestation of dwarf bunt there would be a heavy spore load in the stubble and on the soil surface. In plowing this stubble under, the great bulk of the dwarf bunt spores would be buried to a depth of about 6 inches, where they would remain mostly undisturbed by tillage operations throughout the fallow period. At this depth the spores would be well below the average seeding depth for wheat and too deep for the proper combination of light, aeration, moisture, and temperature to stimulate germination. They would therefore remain inactive during the ensuing crop year, and consequently the incidence of dwarf bunt would be low.

Plowing under the stubble from this crop with a low incidence of dwarf bunt sets the stage for a heavy incidence in the following crop, because plowing returns to the surface a layer of soil containing the heavy spore load from the previous crop. The smutted heads and spore balls thus returned to the surface, further disintegrate from the fallowing and weathering processes, and the spores become widely distributed throughout the surface and sub-surface layers of soil where they are able to germinate. When wheat is seeded in this soil, there is an abundance of effective inoculum at the right depth and the incidence of dwarf bunt in the crop is high. Thus the cycle of heavy dwarf bunt in this case would occur in alternate crop years, or every fourth calendar year.

To actuate an alternate-year cycle in a dwarf-bunt-infested area where the above type of rotation is followed, the rhythm of deep and shallow spores must occur in opposite years on other fields or farms. Still other fields or farms might escape heavy infestations for unknown reasons. The hypothetical operation of this sequence, as it might occur in an alternate year cycle of dwarf bunt in any given locality (4), is shown in Table I. It is assumed that the original infestation increased in prevalence and severity in such a way as to establish the deep and shallow rhythms of spore depth in different fields in opposite years. Thus, while the individual fields

in Groups A and B (Table I) experience high infestations of dwarf bunt only once in four years, the area as a whole exhibits an alternate-year cycle. Since the fields in these groups were in crop and fallow the same years, there must have been a third group of fields (designated as C in Table I) that, for various unknown reasons, either escaped entirely or had only low infestations every crop year. These are the farms on which the wheat crop for the area was produced in the low-incidence years. Apparently this is the only way an alternate-year cycle would develop in an area where the stubble is plowed under.

In areas where the wheat—summer fallow rotation employs a stubble mulch or where the rotation is wheat-peas, an alternate-year cycle could develop in individual fields. Stubble-mulching would tend to keep the spores near the surface where they may serve as effective inoculum each crop year. Obviously, in an area of this kind, as the infestation spreads to other farms, the trend would be toward annual high infestations for the area as a whole. On the other hand, because some spores undoubtedly would germinate during the fallow year, the level of infection might be expected to be

TABLE I. THE PROBABLE SEQUENCE OF EVENTS IN THE OPERATION OF AN ALTERNATE-YEAR CYCLE IN THE INCIDENCE OF DWARF BUNT, AS RELATED TO DEPTH OF SPORES IN THE SOIL IN A WHEAT-FALLOW ROTATION

	Field Groups						
	A			B		C*	
Year	Rotation	Spore Depth	Smut Incidence	Spore Depth	Smut Incidence	Rotation	Smut Incidence
1938	Wheat	Shallow	High	Deep	Low	Fallow	.....
1939	Fallow	Deep	.....	Shallow	.....	Wheat	Nil
1940	Wheat	Deep	Low	Shallow	High	Fallow	.....
1941	Fallow	Shallow	.....	Deep	.....	Wheat	Nil
1942	Wheat	Shallow	High	Deep	Low	Fallow	.....
1943	Fallow	Deep	.....	Shallow	.....	Wheat	Nil
1944	Wheat	Deep	Low	Shallow	High	Fallow	.....
1945	Fallow	Shallow	.....	Deep	.....	Wheat	Nil
1946	Wheat	Shallow	High	Deep	Low	Fallow	.....
1947	Fallow	Deep	.....	Shallow	.....	Wheat	Nil

* The crops in this group of fields escape dwarf bunt infestation for reasons not related to depth of spores in the soil, such as location, farming practices, and the use of smut control measures. Hence, these hypothetical groups are not necessarily always made up of the same fields every year.

relatively low, compared with areas where the stubble is plowed under. This has been observed to be the case in the infested area of Douglas County, Washington, where a wheat-stubble mulch fallow rotation is employed.

Where the rotation involves peas, the infested wheat stubble would be plowed deep and remain undisturbed until after the peas were harvested. To prepare for seeding wheat the pea stubble would be plowed under, and thus the crop of dwarf bunt spores would be returned to shallow depths where they could function to infect the wheat crop. This theoretical sequence of events with respect to dwarf bunt cycles in a wheat-pea rotation has not been substantiated by field observations.

Summary

A possible explanation, based on requirements for spore germination in relation to farming practices, is offered for the cyclic occurrence of dwarf bunt (*Tilletia contraversa* Kühn) of winter wheat in the Pacific Northwest. Soil management, particularly as it relates to the utilization of wheat stubble in soil conservation, may be the key factor in these cycles. Factors affecting spore germination probably also are involved. Dwarf bunt spores germinate best when the proper combination of such factors as temperature, light, moisture, aeration, and possibly others, prevails. Plowing under the dwarf-bunt-infested stubble and surface soil brings the spores into an environment unfavorable for their germination, primarily because of inadequate aeration and the absence of light. Here, presumably, the spores remain dormant until the next plowing returns them to the surface, where optimum germination conditions prevail. Wheat crops grown while the spores remain deep in the soil may escape severe infestation while crops grown when the spores are on or near the surface may become heavily infested. Hence, the nature of the cyclic occurrence of dwarf bunt in any given infested area may be related to the schedule of deep plowing.

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MORPHOLOGY AND CYTOLOGY OF SPORE GERMINATION AND SUBSEQUENT DEVELOPMENT OF *USTILAGO SPEGAZZINII* VAR. *AGRESTIS* IN CULTURE*

SHERL M. DIETZ†

INTRODUCTION

At the outset it is necessary to clarify the species concept of the stem smut fungi. *Caeoma hypodytes* Schlechtendal, the first species of this group described (23), was transferred to the genus *Ustilago* by Fries in 1832. In 1945 Fischer and Hirschhorn (13) made a critical study of material of *U. hypodytes* issued as authentic exsiccati by many prominent European mycologists as well as extensive American collections. They concluded that the binomial *U. hypodytes* (Schlecht.) Fries was a *nomen dubium* representing a complex of smut fungi, embracing four species and one variety. These were separated into two groups: (1) species whose spores possess bipolar areas or appendages [*U. spegazzinii* Hirschh. and its variety *agrestis* (Syd.) G. W. Fisch. & Hirschh. and *U. williamsii* (Griff.) Lavrov.] and (2) species whose spores lack bipolar areas or appendages (*U. halophila* Speg. and *U. nummularia* Speg.). *U. nummularia* was later reduced to synonymy with *U. hypodytes*. Thus they differentiated *U. spegazzinii* from the other three species of stem smut fungi occurring in the *U. hypodytes* complex.

Although several studies on the germination of European material of *Ustilago hypodytes* have been published (2, 3), only limited data are presented for American specimens of *U. spegazzinii* var. *agrestis* (18). This paper will present studies of the effects of the medium and temperature upon germination of teliospores of *U. spegazzinii* var. *agrestis*. The subsequent morphological and cytological development of the fungus in culture and studies of teliospore longevity will be considered.

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Effect of Medium and Temperature Upon Germination

In 1883 Brefeld (4) reported that spores of *U. hypodytes (=U. spegazzinii?)* germinate readily in water. Bornhövd (2) obtained 1-10 per cent germination in tap and distilled water. Since the organism produced sparse, abnormally twisted mycelium and no sporidia in water, this method of germination was not satisfactory. A study was therefore undertaken using six agar media in Petri dishes, as follows (per liter):

1. Potato-sucrose agar (PSA)
   200g fresh potatoes
   10g sucrose
   4g asparagine
   1g yeast extract
   20g agar
2. Potato-dextrose agar (PDA)
   200g fresh potatoes
   10g dextrose
   4g asparagine
   1g yeast extract
   20g agar
3. Potato-sucrose agar
   200g fresh potatoes
   10g sucrose
   20g agar
4. Difco potato-dextrose agar
   39g Difco dehydrated PDA
   15g agar
5. Difco potato-sucrose agar
   400g dehydrated potatoes
   60g sucrose
   1g peptone
   20g malt extract broth
   20g agar
6. Difco potato-dextrose agar
   30g dehydrated PDA
   9g agar
   4g asparagine
   1g yeast extract

The medium in each Petri plate was divided into three sectors. Each sector was streaked with a loop of teliospores from a different host *(Agropyron repens, Elymus cinereus,* and *E. glaucus)*. This streaking was done in duplicate for all six media. One set each of the Petri plates was incubated at 25° C. and at 30° C. This experiment was conducted twice with two replicates in the first run and three in the second. Spore germination counts were made after 21 hours of incubation. Average germination percentages for all replicates are shown in Table I.

TABLE I. Teliospore Germination of Ustilago spegazzinii var. agrestis after 21 Hours on Six Different Media at 25° C. and 30° C.

Medium	Average Per Cent Germination in 21 Hours	
	25° C.	30° C.
1. PSA	43	55
2. PDA	26	39
3. PSA	25	38
4. PDA (Difco) (3% agar)	18	27
5. PSA (Difco)	trace	trace
6. PDA (Difco)	0	0

Germination in 21 hours was good on the first two media. However, these media promoted mycelial growth to the exclusion of sporidial production. In later studies germination on medium 4 was improved by reducing the agar content from 3 to 2 per cent. Subsequent sporidial production was likewise better on the 2 per cent agar. Since media 3 and 4 (with 2 per cent agar) are simple to prepare, are easy to duplicate, and promote the desired sporidial growth, they were used in later temperature studies. For each medium, 9-13 per cent higher germination was obtained in 21 hours at 30° C. than at 25° C., except where only a trace or no germination was observed.

A temperature series ranging from 5° C. to 35° C. at 5° increments was used to determine cardinal temperatures. The plates were inoculated as described previously. Germinated teliospore counts were made at regular intervals from 18 to 250 hours. The percentage of teliospore germination for the three collections after incubation for 48 hours at the various temperatures is shown in Table II.

TABLE II. PER CENT TELIOSPORE GERMINATION FOR THREE COLLECTIONS OF USTILAGO SPEGAZZINII VAR. AGRESTIS AT VARIOUS TEMPERATURES C. IN 48 HOURS ON 2 PER CENT POTATO-DEXTROSE AGAR

Host	5°	10°	15°	20°	25°	30°	35°
A. repens	0	0	4	13	67	72	0
E. glaucus	0	0	0	34	85	96	0
E. cinereus	0	0	trace	14	66	72	0

The 48-hour period was chosen to best indicate the temperature effect on germination, since germination at temperatures from 15-25° C. will eventually reach a maximum equal to that obtained at 30° C.

Readings after 48 hours of incubation indicate that best germination was obtained at 30° C. Slightly less occurred at 25° C. and still less at 20° C. Little germination was obtained at 15° C. After approximately one month no germination had occurred at 5° C. or at 35° C. However, good cultures were obtained when these spores were placed at 30° C., proving that they were still viable. At the optimum temperature (30° C.), germination commenced in 18 to 20 hours on 2 per cent potato-dextrose agar. This is approximately half the 40 or more hours previously reported for this organism by Fischer and Hirschhorn (13), using potato-dex-

trose agar at room temperature (20-22° C.), but only slightly less than the 24 hours or more reported by Fischer (9). Germination commenced after 129 hours at 10° C.

In addition to the slow growth at this temperature, it was noticed that the lateral branches from the promycelium were shorter and sporidia appeared to bud from their tips. However, sporidia were never observed to bud directly off the promycelium as in *Ustilago avenae* and other smut fungi. This cold temperature effect is similar to the results obtained by Hüttig (20) with *U. nuda.* One hundred per cent germination was never observed, even with fresh collections, but was approached (e.g., 96 per cent at 48 hours for teliospores from *Elymus glaucus).*

Although Bornhövd (2) reported the cardinal temperatures for *U. hypodytes (=U. spegazzinii)* as minimum 10° C., optimum 26-29° C., and maximum 34-35° C., the results of these studies tend to indicate 10° C., 30° C., and lower than 35° C.

Bipolar Areas

The principal diagnostic characteristics of the teliospores of *Ustilago spegazzinii* and its variety *agrestis* are the differentiated bipolar areas (Fig. 1A & B). In a recent study of some 250 specimens from Europe and America, Angus (1) reports that the possession of these "caps" is not of sufficient morphologic significance or consistency to consider *U. spegazzinii* a distinct species. Thus he reduces it to synonymy with *U. hypodytes* (Schlecht.) Fries. Angus (1), in agreement with our observations, finds the bipolar areas visible only when the teliospore poles are oriented at a suitable angle to the line of vision.

In a lactophenol cotton-blue mount of *U. spegazzinii* var. *agrestis* from *Elymus cinereus,* only 30 per cent of the spores appeared to possess the bipolar areas; but out of 100 which appeared to lack them, 94 revealed them when rolled over by gently tapping the cover-slip. Conversely, those with the bipolar areas in view could be rolled to a position concealing the areas. Similarly treated material from *Agropyron repens* showed the areas on only approximately 38 per cent of the teliospores; but when bleached in 5 per cent sodium hypochlorite and stained with crystal violet (Fig. 1C), nearly 100 per cent of the teliospores exhibited these "cap" areas. Although speciation probably should not be based on this histological method, nevertheless the evidence substantiates the presence of

caps on nearly all the spores of the collections thus stained. This method makes it unnecessary to orient a spore in order to detect the presence of bipolar areas.

Teliospore Germination

A study of germinated teliospores of *Ustilago spegazzinii* var. *agrestis* stained with haematoxylin following the method used by Hirschhorn (18) indicates that the promycelium almost always emerges at the side of or occasionally directly through one of these crests (Fig. 1D & E). Rarely does germination occur through both polar areas, producing two promycelia from the same teliospore (Fig. 3A & B). It is believed that the polar areas essentially function as germ pores.

Attempts to germinate several fresh collections of stem smut identified by G. W. Fischer as *U. hypodytes* (no bipolar areas) under the same conditions employed in germinating *U. spegazzinii* var. *agrestis,* failed. Many earlier workers, Winter (24), Brefeld (4), Essmon (6), Boss (3) and Bornhövd (2), were able to germinate teliospores of material they identified as *U. hypodytes.* Others, including Fischer v. Waldheim (15), Plowright (22), and Winter (24), were unable to germinate teliospores of material similarly identified. It is of special interest that Winter (24), who gave a description and illustrated the germination of *U. hypodytes,* later reported he was unable to germinate teliospores of this species. Current results suggest one possible explanation of this anomalous situation: The binomial *U. hypodytes* (Schlecht.) Fries, as previously used, included several species, later described by Fischer and Hirschhorn (13), differing in germinability as well as morphology (oil immersion). However, Fischer and Hirschhorn (13) did observe the germination of *U. nummularia,* now a synonym of *U. hypodytes,* but found it germinated more slowly (two days or more) and produced a mycelial growth on which sporidia were never observed.

In examining teliospores from many specimens, it is recognized that variations in the extent of the bipolar areas do exist. Whether these differences in spore morphology and germination are enough to maintain two separate and distinct species, or whether it would be better to enlarge the original description of *Ustilago hypodytes* to include forms with bipolar areas and thus reduce *U. spegazzinii* and its variety *agrestis* to synonymy, remains to be determined.

Cytological Studies

A schedule with Flemming's weak solution and haematoxylin similar to that of Hirschhorn (18) was utilized for the nuclear study.

According to Bornhövd (2), DeBary in 1853 claimed that the nucleus appeared to fill the entire teliospore. Results of this study confirm those of Bornhövd (2), who reported that the teliospore fusion nucleus is approximately ¼ to ½μ (Fig. 1B). As previously stated, the teliospores germinate in 18 or more hours on 2 per cent potato-dextrose agar or potato-sucrose agar at 30° C. The promycelium emerges at the side of or occasionally directly through one of the bipolar areas (Fig. 1D & E). Generally, by the time the promycelium has grown in length to approximately twice the teliospore diameter, the fusion nucleus has migrated into the promycelium. Bornhövd (2) and Hirschhorn (18) report occasional divisions of the fusion nucleus within the teliospore. These divisions were not observed in my preparations.

The fusion nucleus is shown migrating from the teliospore in Figure 1G upper left. In the upper center it is seen well out into the promycelium. The fusion nucleus divides (Fig. 1F), and a septum is usually formed soon thereafter (Fig. 1G, lower right). Hirschhorn (18) stated that the first metaphase plate contained four chromosomes. What appeared to be division figures were seen in this study, but nothing was observed that could be interpreted with certainty as individual chromosomes. The two daughter nuclei of the first division divide, producing a four-nucleate two-celled promycelium (Fig. 1H). Two more septations may appear, resulting in a promycelium with four cells, each with its own nucleus. Usually only one additional septum is formed near the base of the promycelium, resulting in a four-nucleate three-celled promycelium (Fig. 1I). Instead of budding sporidia directly, the elongating promycelium produces lateral branches similar to *Ustilago nuda* (Fig. 1I & J). Typically, the terminal promycelial cell elongates and remains aseptate for some time while the lower cells each produce two and even three branches (Fig. 1J).

In general these observations confirm those of Bornhövd (2). A promycelial nucleus may migrate into a branch, but usually nuclear division occurs within the promycelial cell. One of the daughter nuclei then migrates into the newly formed branch; the other

Figure 1. (A-J) teliospore material of *Ustilago spegazzinii* var. *agrestis* obtained from *Agropyron repens*: (AB) teliospores with equatorial band and bipolar areas; (B) fusion nucleus within ungerminated teliospore; (C) teliospore bipolar areas stained with crystal violet subsequent to 5 per cent sodium hypochlorite treatment; (D-E) germinating teliospores with the promycelium emerging through or at the side of a bipolar area and the fusion nucleus moving out into the promycelium; (F) first division of the fusion nucleus in the promycelium; (G) upper left, fusion nucleus migrating into the promycelium, upper center, fusion nucleus well out into promycelium, lower right, binucleate, two-celled promycelium; (H) four nucleate, two-celled promycelium; (I) four nucleate but three-celled promycelium beginning to branch; (J) promycelium with six lateral branches.

Figure 2. (A-C) *Ustilago spegazzinii* var. *agrestis* from *Agropyron repens* in culture. (A) Typical germination of two teliospores on 2 per cent potato dextrose agar. The one on the left has branched extensively; (B) mycelial mounding and roping; (C) clusters of aerial sporidia.

Figure 3. (A-I) *Ustilago spegazzinii* from *Agropyron repens* in culture. (A-B) Germination from both bipolar areas; (C-I) production of aerial sporidia; (C) short, erect, peg-like sporidiophores commence to arise from the mycelial mat below; (D) haematoxylin-stained preparation of a binucleate aerial sporidiophore, with one nucleus migrating into the newly produced vesicle; (E) beginning of sporidial formation; (F) aerial sporidiophore and vesicle upon which are borne short chains of sporidia and another vesicle; (G) branched aerial sporidiophore and sporidia; also robust infection hypha resulting from a hyphal anastomosis; (H) enlargement of single vesicle and short chains of sporidia; (I) nuclei of aerial sporidia stained with Giemsa.

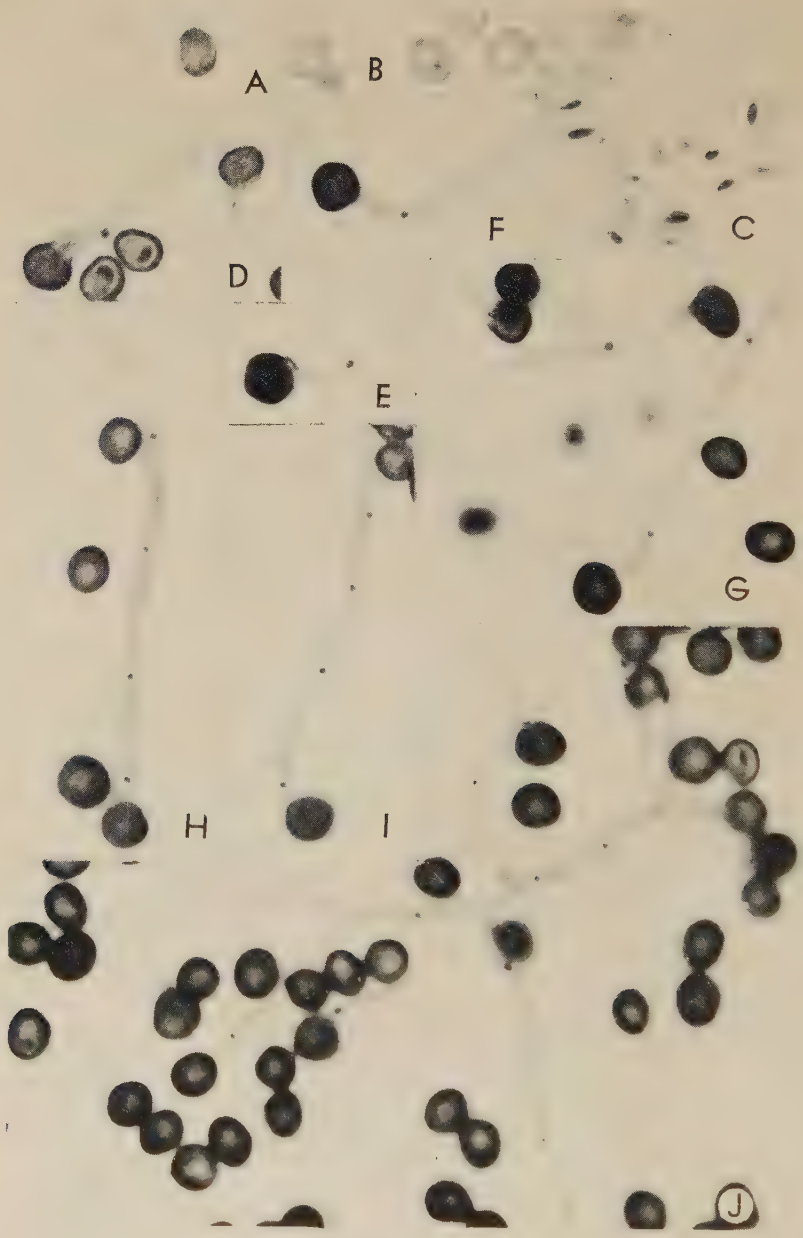

Figure 1

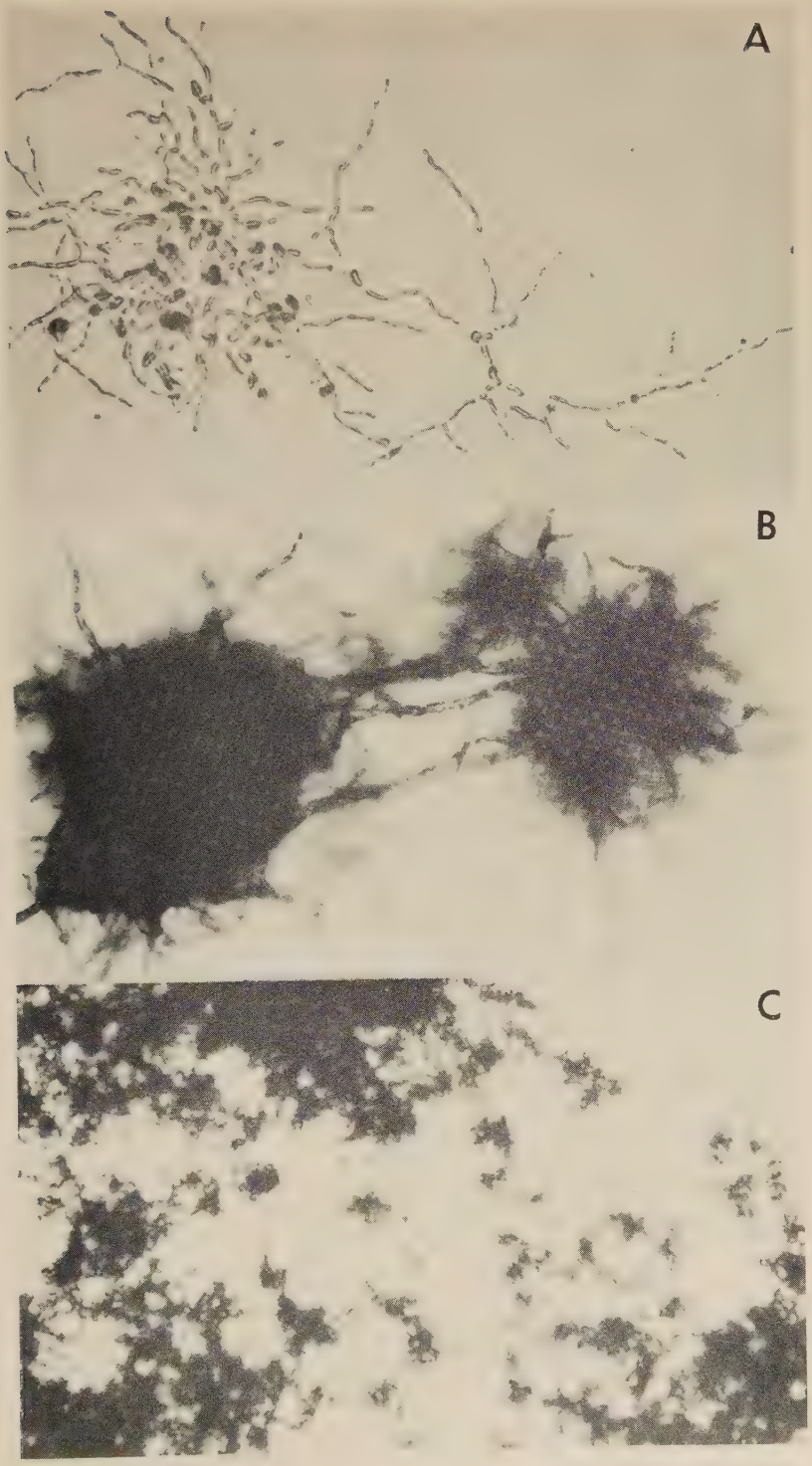

Figure 2

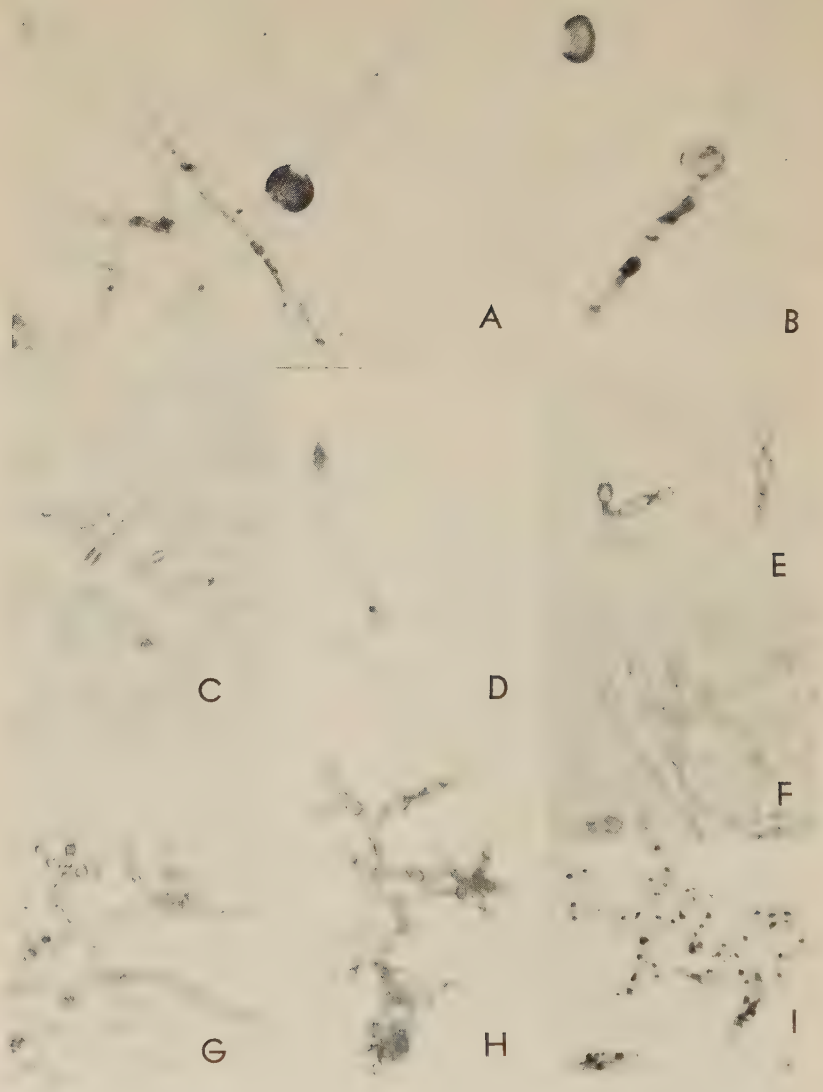

Figure 3

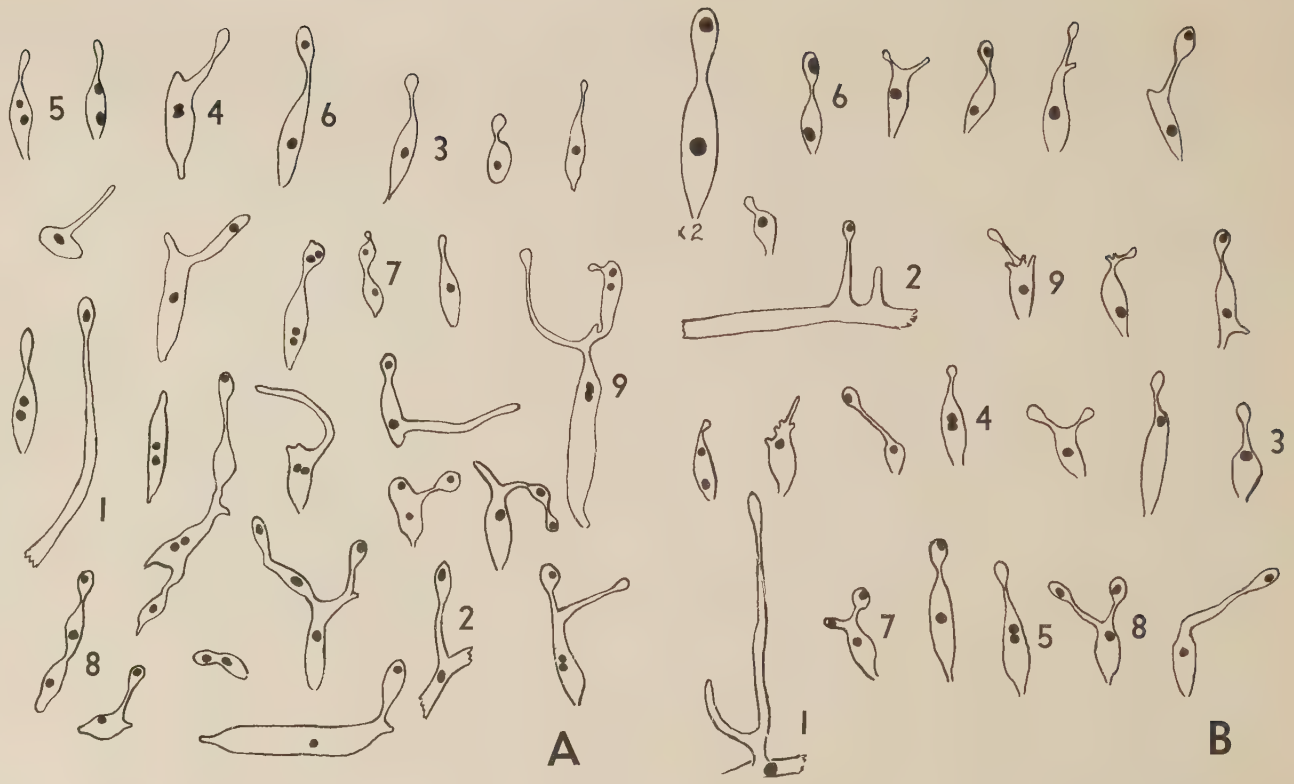

Figure 4. Camera lucida drawings illustrating the nuclear condition of aerial sporidia of *Ustilago spezazzinii* var. *agrestis* (obtained from (A) Elymus glaucus and (B) *Elymus cinereus*).

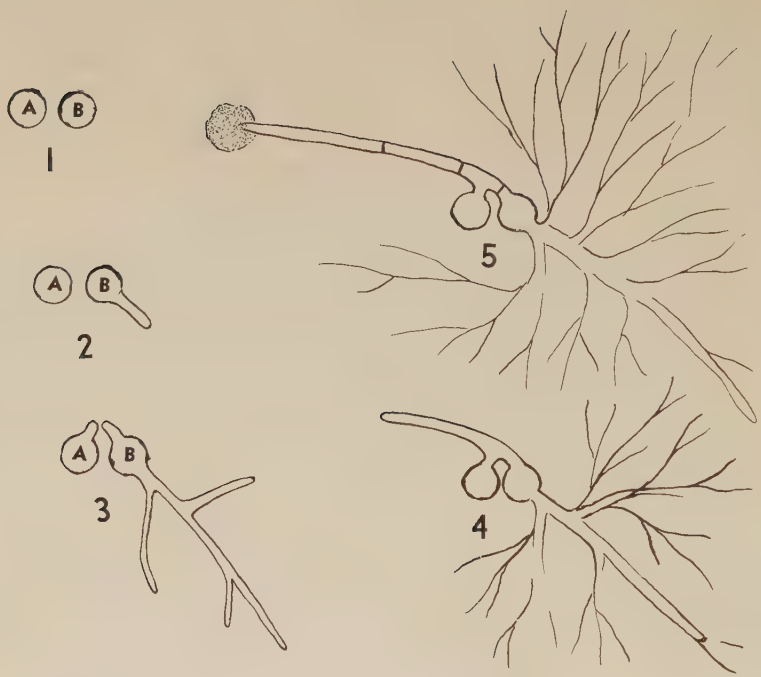

Figure 5. A five-step diagrammatical drawing illustrating sporidial fusion and subsequent development of the infection hypha. Also shown is the atypical development of a ramifying mycelium which produced aerial sporidia. (1) Sporidia (A & B) from different sporidiophores placed in close proximity on 3% water agar. (2) Sporidium B germinated. (3) Sporidium A germinated and sporidium B produced a second germ tube which (4) fused with that of sporidium A resulting in an infection hypha, while the first germ tube produced had branched and rebranched forming a ramifying mycelium which later produced aerial sporidia. (5) The protoplasm moved out into the advancing tip of the infection hypha and laid down three septa behind. The infection hypha ruptured at its apex, releasing its protoplasmic contents onto the agar.

remains in the promycelium. Further nuclear divisions and septations result in a uninucleate ramifying mycelium. As the mycelium develops, the protoplasm moves out into the advancing hyphal tips, often walling off empty cells behind. Structures resembling clamps have been observed in culture on these advancing hyphal tips, but stained preparations have not confirmed this observation.

In growing this fungus under non-optimal conditions, several other types of germination were observed. On 3 per cent water agar the promycelium often produces "knee-joints", indicating fusion between two promycelial cells. On a rich nutrient medium sporidia may be formed at the tips of primary branches from the promycelium, as pictured by Fischer and Hirschhorn (13). Also, as previously mentioned, when this organism is grown at 10° C. the promycelial branches are very short and the sporidia bud off from their tips.

Rarely, germination occurs from both "crests", producing two promycelia from the same teliospore (Fig. 3A & B). In these cases, however, the nuclear phenomena could not be ascertained. However, it appears that when two promycelia are produced, although both are functional, one predominates in size and vigor (Fig. 3A & B).

The typical branching promycelial growth on 2 per cent potato-dextrose agar is illustrated in Figure 2A. At the left, branching and rebranching from a single germinated teliospore has developed extensive mycelial growth which is beginning to mound up. This mounding of mycelium may continue to the point of forming mycelial ropes consisting of aggregates of from a few to several hundred entwined hyphae (Fig. 2B). These may become so large they are readily visible to the unaided eye. On 2 per cent Difco potato-dextrose agar both this mycelial mounding and aerial sporidial formation occur, but usually one predominates. Generally, clumps of aerial sporidia are produced, appearing as a white powdery growth on the medium (Fig. 2C).

Production of Aerial Sporidia

Numerous short erect peg-like hyphae arise from the mycelial mat (Fig. 3C). These become aerial sporidiophores which enlarge

apically, forming vesicles.[1] The nucleus divides in the sporidiophore. One nucleus then migrates into the newly formed vesicle (Fig. 3D and Fig. 4A1 & 2, B1 & 2). This vesicle then forms from one to as many as six minute sterigma-like processes at its apex, from which swellings arise and enlarge into sporidia. Camera lucida drawings of sporidial production and the associated nuclear phenomena are shown in Figure 4A & B. The terminal sporidium again forms a minute sterigma-like projection, from the end of which a swelling arises and gradually enlarges (Fig. 4A3, B3). By the time this swelling is approximately half the size of the parent sporidium, nuclear division takes place (Fig. 4A4 & 5, B4 & 5). One daughter nucleus migrates into the newly formed sporidium (Fig. 4A6, B6). This repetitive process (Fig. 4A7 & 8, B7 & 8) forms short sporidial chains (Fig. 3E, F, G, H). Thus the mycelium, sporidiophores, and sporidia (Fig. 3I) are all monocaryotic, as observed by Bornhövd (2) and Boss (3). Giemsa stain (16) was used in the nuclear investigations of sporidia.

Short aerial chains of sporidia adhere tenaciously to their erect sporidiophores. They could not be blown off while being observed through a microscope, but were readily dispersed by the addition of water or other liquids. The sporidia or the mycelium are extremely resistant to desiccation. Cultures allowed to dry completely on agar for more than one year could still be revived by transfer to fresh medium.

Single sporidia were isolated by means of a glass needle mounted on a Chambers micromanipulator. Each individual sporidium was then transferred to a small block of nutrient medium and placed in a 30° C. incubator. In some cases germination occurred overnight (15 hours or less). Each sporidium produced one and often two germ tubes which, by much rebranching, formed a ramifying mycelium resembling that resulting from teliospore germination. From this mycelium, aerial sporidiophores with short chains of sporidia soon arose. These sporidiophores and their sporidia were morphologically indistinct from those formed from teliospores.

[1] For the want of a better term these structures were at first called basal cells, because they form a base from which subsequent sporidia are produced. However, in describing their development one had to state that the *basal* cell was formed by an *apical* swelling of the sporidiophore. In an attempt to circumvent this ambiguity, the term vesicle is used. The term vesicle as used in *Aspergillus* and *Penicillium* is applied to a bladder-like structure at the apex of a conidiophore which gives rise to phialides that in turn support the conidia. In this smut organism, the vesicle may give rise directly to the sporidia or to more vesicles (Fig. 3F).

Examination of many sporidial matings on nutrient media failed to disclose any fusion. Each sporidium of the pair germinated independently and produced aerial sporidia, as previously described. Occasional fusions were observed on water agar when sporidia from two different sporidiophores were mated. Mated sporidia isolated from the same sporidiophore have not been observed to fuse.

One particularly interesting observation is shown diagrammatically in five steps (Fig. 5). This diagram illustrates an atypical case in that usually only a single infection hypha develops from a fusion between compatible sporidia. Using a micromanipulator, sporidia A and B obtained from different sporidiophores were placed very close to one another on a small block of water agar (step 1). Sporidium B germinated first from the side facing away from sporidium A (step 2). After this germ tube lengthened and branched, sporidium A germinated and sporidium B sent out a second germ tube in the direction of sporidium A (step 3). The germ tube from sporidium A then fused with the second germ tube produced by sporidium B (step 4). By this time the first germ tube from sporidium B had branched extensively and formed a ramifying mycelium with erect sporidiophores commencing to form. The fusion tube became long and eventually ruptured at its apex, releasing its protoplasmic contents on the agar in a manner similar to the infection hyphae of *Tilletia caries* on agar (step 5). However, the mycelium resulting from the first germ tube of sporidium B continued to grow and produce aerial sporidia in the same manner as monosporidial isolates.

Hyphal anastomoses were observed which could not be pulled apart with the aid of the micromanipulator. A robust aerial hypha (Fig. 3G, bottom right) grows rapidly from the junction of the hyphal anastomosis. Bornhövd (2) demonstrated this to be unicellular and binucleate. She also demonstrated that in the absence of a suitable host the dicaryon reverts to the monocaryotic stage and produces more aerial sporidia.

The dicaryophase thus is initiated in three ways: (1) fusion between promycelial cells, (2) fusion between sporidia from different sporidiophores, and (3) hyphal anastomoses.

Longevity Studies

Incidental to the spore germination studies, observations were made on spore longevity. Spore material was used from 48 col-

lections of *Ustilago spegazzinii* and its variety *agrestis* ranging in age from 2 to 14 years. These collections embraced eight grass genera represented by 12 species of *Agropyron,* five of *Elymus,* four of *Poa,* two of *Sitanion,* and one each of *Phalaris, Oryzopsis, Melica,* and *Puccinellia,* making a total of 27 different grass hosts. Most of the specimens were obtained in the vicinity of Pullman, Washington, but some were from Oregon, Nevada, Utah, and California.

Germination studies were conducted by streaking spores of each collection with a sterile loop needle onto poured 2 per cent potato-dextrose agar in Petri plates, and incubating at room temperature (20-24° C.). Those failing to germinate within two weeks were considered non-viable under the conditions of this experiment. Teliospore germination dropped markedly in collections older than 3 years. Three out of five of the 6-year-old collections germinated, but only four out of ten of the 7-year-old collections. Since all ten of the 7-year-old collections were from different hosts, it was impossible to ascertain whether the host plant or maturity of the teliospores at the time of collection was of greater significance as a factor in longevity. However, spores from a 6-year-old collection from *Agropyron trachycaulum* failed to germinate, whereas germination was obtained in the case of a 7-year-old collection from the same host.

This would tend to support Fischer's (7) opinion that within a species the state of maturity at the time of collection determines, in large measure, the longevity of spores. Teliospore development of this stem smut organism occurs progressively from the base of the culm internode and centripetally within the spore layer. Thus the oldest spores are on the surface of the spore layer at the top of the internode. Whenever possible, spores were obtained from this area.

All 23 collections more than 7 years old failed to germinate with one notable exception, viz., 12-year-old teliospore material obtained from *Agropyron trachycaulum.* In 1938, at the time this material was originally collected, several grams of teliospores were scraped from the culms and placed in a small, tightly-sealed screw-cap glass jar. All other collections were packeted and filed. The fact that spores of this tightly-sealed collection were still viable after 12 years suggests that the method of storage, as well as stage of maturity, may be important in longevity.

Summary

1. Teliospores of three collections of *Ustilago spegazzinii* var. *agrestis,* obtained from *Agropyron repens, Elymus cinereus,* and *E. glaucus,* were germinated on six different media. Two per cent agar formulations of Difco potato-dextrose agar and potato-sucrose agar gave ample germination and supported abundant sporidial production.
2. Using the same three smut collections and media mentioned above, the cardinal temperatures were determined to be: minimum 10° C., optimum 30° C., and maximum less than 35° C. At the optimum temperature germination commenced at 18 hours.
3. When stained with crystal violet following a sodium hypochlorite treatment, nearly 100 per cent of the teliospores of *U. spegazzinii* var. *agrestis* from *Agropyron repens, Elymus cinereus,* and *E. glaucus* were found to possess differentiated bipolar areas.
4. Cytological studies on teliospore germination and subsequent sporidial production, essentially confirm the results of Boss (3), Bornhövd (2), and partly those of Hirschhorn (18). Teliospores germinate by way of the bipolar areas and generally produce a four-nucleate three-celled promycelium. Lateral branches soon arise from the promycelium similar to *U. nuda.* These laterals also branch and rebranch to form a ramifying mycelium upon which sporidiophores develop bearing short chains of aerial sporidia. The individual promycelial cells, the resulting mycelium, and the aerial sporidia are all uninucleate. Detached sporidia may germinate overnight (15 hours or less), often producing two germ tubes which branch extensively to form a ramifying mycelium and more aerial sporidia, constituting a secondary sporidial cycle. Sporidia isolated from the same sporidiophore and mated on water agar did not fuse. Compatible sporidia from different sporidiophores did fuse.
5. Initiation of the dicaryon occurs by three methods: (1) fusion between promycelial cells, (2) hyphal anastomosis, and (3) fusion between compatible sporidia obtained from different sporidiophores.
6. Teliospore longevity studies indicate that germination drops markedly in collections older than 3 years, with some collec-

tions failing to germinate. Of 23 collections older than 7 years, only one 12-year-old collection germinated. Both spore maturity at the time of collection and method of storage are believed to be important in longevity.

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